

This Page Is Inserted by IFW Operations
and is not a part of the Official Record

BEST AVAILABLE IMAGES

Defective images within this document are accurate representations of the original documents submitted by the applicant.

Defects in the images may include (but are not limited to):

- BLACK BORDERS
- TEXT CUT OFF AT TOP, BOTTOM OR SIDES
- FADED TEXT
- ILLEGIBLE TEXT
- SKEWED/SLANTED IMAGES
- COLORED PHOTOS
- BLACK OR VERY BLACK AND WHITE DARK PHOTOS
- GRAY SCALE DOCUMENTS

IMAGES ARE BEST AVAILABLE COPY.

**As rescanning documents *will not* correct images,
please do not report the images to the
Image Problem Mailbox.**

PCT

WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

AM

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

| | | | | | |
|---|--|--|--|--|--|
| (51) International Patent Classification ⁶ : A61K 38/18 | | A1 | (11) International Publication Number: WO 97/41881 (43) International Publication Date: 13 November 1997 (13.11.97) | | |
| (21) International Application Number: | PCT/US97/07816 | (81) Designated States: | AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TR, TT, UA, UG, UZ, VN, YU, ARIPO patent (GH, KE, LS, MW, SD, SZ, UG), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG). | | |
| (22) International Filing Date: | 6 May 1997 (06.05.97) | (30) Priority Data: | 08/643,321 6 May 1996 (06.05.96) US | | |
| (71) Applicant: | CREATIVE BIOMOLECULES, INC. [US/US]; 45 South Street, Hopkinton, MA 01748 (US). | (72) Inventors: | SAMPATH, Kuber, T.; 6 Spring Street, Medway, MA 02053 (US). COHEN, Charles, M.; 1 Harrington Lane, Weston, MA 02193 (US). | | |
| (74) Agent: | (74) Agent: TWOMEY, Michael, J.; Testa, Hurwitz & Thibeault, LLP, High Street Tower, 125 High Street, Boston, MA 02110 (US). | | | | |
| (54) Title: MORPHOGEN TREATMENT FOR CHRONIC RENAL FAILURE | | Published <i>With international search report. Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i> | | | |
| (57) Abstract | | | | | |
| <p>The present invention provides methods for the treatment, and pharmaceuticals for use in the treatment, of mammalian subjects in, or at risk of, chronic renal failure, or at risk of a need for renal replacement therapy. The methods involve the administration of certain proteins of, or based upon, the osteogenic protein/bone morphogenetic protein (OP/BMP) family of the TGF-β superfamily of proteins, or the administration of certain morphogens, inducers of those morphogens, agonists of the corresponding morphogen receptors, or implantation of renal cells induced with those morphogens. The morphogens useful in the invention are also members of, or based upon, the OP/BMP family of proteins.</p> | | | | | |

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

| | | | | | | | |
|----|--------------------------|----|---------------------------------------|----|---|----|--------------------------|
| AL | Albania | ES | Spain | LS | Lesotho | SI | Slovenia |
| AM | Armenia | FI | Finland | LT | Lithuania | SK | Slovakia |
| AT | Austria | FR | France | LU | Luxembourg | SN | Senegal |
| AU | Australia | GA | Gabon | LV | Latvia | SZ | Swaziland |
| AZ | Azerbaijan | GB | United Kingdom | MC | Monaco | TD | Chad |
| BA | Bosnia and Herzegovina | GE | Georgia | MD | Republic of Moldova | TG | Togo |
| BB | Barbados | GH | Ghana | MG | Madagascar | TJ | Tajikistan |
| BE | Belgium | GN | Guinea | MK | The former Yugoslav Republic of Macedonia | TM | Turkmenistan |
| BF | Burkina Faso | GR | Greece | ML | Mali | TR | Turkey |
| BG | Bulgaria | HU | Hungary | MN | Mongolia | TT | Trinidad and Tobago |
| BJ | Benin | IE | Ireland | MR | Mauritania | UA | Ukraine |
| BR | Brazil | IL | Israel | MW | Malawi | UG | Uganda |
| BY | Belarus | IS | Iceland | MX | Mexico | US | United States of America |
| CA | Canada | IT | Italy | NE | Niger | UZ | Uzbekistan |
| CF | Central African Republic | JP | Japan | NL | Netherlands | VN | Viet Nam |
| CG | Congo | KE | Kenya | NO | Norway | YU | Yugoslavia |
| CH | Switzerland | KG | Kyrgyzstan | NZ | New Zealand | ZW | Zimbabwe |
| CI | Côte d'Ivoire | KP | Democratic People's Republic of Korea | PL | Poland | | |
| CM | Cameroon | KR | Republic of Korea | PT | Portugal | | |
| CN | China | KZ | Kazakhstan | RO | Romania | | |
| CU | Cuba | LC | Saint Lucia | RU | Russian Federation | | |
| CZ | Czech Republic | LJ | Liechtenstein | SD | Sudan | | |
| DE | Germany | LK | Sri Lanka | SE | Sweden | | |
| DK | Denmark | LR | Liberia | SG | Singapore | | |

MORPHOGEN TREATMENT FOR CHRONIC RENAL FAILURE

Field of the Invention

The present invention relates generally to methods of treatment for renal disease. In particular, the invention relates to methods of treatment for conditions which place mammals, including humans, in, or at risk of, chronic renal failure. The methods preferably involve the administration of certain proteins of the osteogenic protein/bone morphogenetic protein (OP/BMP) family within the TGF- β superfamily of proteins. More generally, the methods involve the administration of certain morphogens, inducers of those morphogens, or agonists of the corresponding morphogen receptors, or implantation of renal cells induced with those morphogens.

10 Background of the Invention

The mammalian renal system serves primary roles both in the removal of catabolic waste products from the bloodstream and in the maintenance of fluid and electrolyte balances in the body. Renal failures are, therefore, life-threatening conditions in which the build-up of catabolites and other toxins, and/or the development of significant imbalances in electrolytes or fluids, may lead to the failure of other major organs systems and death. As a general matter, renal failure is classified as "acute" or "chronic." As detailed below, the differences between these two conditions are not merely a matter of severity or rapidity but, rather, reflect differences in etiology, prognosis, and treatment.

15 Acute Renal Failure

20 Acute renal failure is defined as an abrupt cessation or substantial reduction of renal function and, in as many as 90-95% of cases, may be secondary to trauma, surgery or another acute medical condition. Acute renal failure may be due to pre-renal causes (e.g., decreased cardiac output, hypovolemia, altered vascular resistance) or to post-renal causes (e.g., obstructions or constrictions of the ureters, bladder or urethra) which do not directly involve the kidneys and which, if treated quickly, will not entail significant loss of nephrons or other damage to the kidneys. Alternatively, acute renal failure may be due to intrinsic renal causes which involve a more direct insult or injury to the kidneys, and which may entail permanent damage to

- 2 -

the nephrons or other kidney structures. Intrinsic causes of acute renal failure include but are not limited to infectious diseases (e.g., various bacterial, viral or parasitic infections), inflammatory diseases (e.g., glomerulonephritis, systemic lupus erythematosus), ischemia (e.g., renal artery occlusion), toxic syndromes (e.g., heavy metal poisoning, side-effects of antimicrobial treatments or chemotherapy), and direct traumas.

The diagnosis and treatment of acute renal failure is as varied as its causes. In human patients, oliguria (urine output < 400 ml/day) or anuria (urine output < 50 ml/day) may be present in 50-70% of cases, BUN levels may climb 10-20 mg/dL/day or faster, plasma creatinine levels may climb 0.5-1.0 mg/dL/day, and metabolic acidosis is almost always present. If not treated, the electrolyte and fluid imbalances (e.g., hyperkalemia, acidosis, edema) associated with acute renal failure may lead to life-threatening arrhythmia, congestive heart failure, or multiple organ system failures. Present therapies are typically directed at the underlying causes of the acute renal failure (e.g., pre-renal, post-renal, or infectious causes) and management of the complications. Due to the severity of acute renal failure, episodes rarely last longer than several weeks without mortality and are treated on an in-patient basis.

Chronic Renal Failure

Chronic renal failure may be defined as a progressive, permanent and significant reduction of the glomerular filtration rate (GFR) due to a significant and continuing loss of nephrons. Chronic renal failure typically begins from a point at which a chronic renal insufficiency (i.e., a permanent decrease in renal function of at least 50-60%) has resulted from some insult to the renal tissues which has caused a significant loss of nephron units. The initial insult may or may not have been associated with an episode of acute renal failure. Irrespective of the nature of the initial insult, chronic renal failure manifests a "final common path" of signs and symptoms as nephrons are progressively lost and GFR progressively declines. This progressive deterioration in renal function is slow, typically spanning many years or decades in human patients, but seemingly inevitable.

The early stage of chronic renal failure typically begins when GFR has been reduced to approximately one-third of normal (e.g., 30-40 ml/min for an average human adult). As a result of the significant nephron loss, and in an apparent "attempt" to maintain the overall GFR with fewer nephrons, the average single nephron GFR (SNGFR) is increased by adaptations of the remaining nephrons at both the structural and functional level. One structural manifestation of this adaptation, readily detectable by microscopic examination of biopsy samples, is a

- 3 -

"compensatory hypertrophy" of both the glomeruli and the tubules of the kidney, a process which literally increases the volume of filtrate which can be produced by each remaining nephron by literal enlargement of the glomeruli and tubules. Indeed, as a result of the hypertrophy or dilation of the collecting ducts, the urine of subjects with chronic renal failure often contains broad

- 5 "casts," typically 2-6 times normal diameter, which aid in diagnosis and have also been referred to as "renal failure casts." At the same time, there are functional changes in the remaining nephrons, such as decreased absorption or increased secretion of normally excreted solutes, which may be responses to hormonal or paracrine changes elsewhere in the body (e.g., increasing levels of parathyroid hormone (PTH) in response to changes in serum levels of calcium and phosphate).

10 These adaptations in early stage chronic renal failure are not successful in completely restoring GFR or other parameters of renal function and, in fact, subject the remaining nephrons to increased risk of loss. For example, the increased SNGFR is associated with mechanical stresses on the glomerulus due to hypertension and hyperperfusion. The loss of integrity of podocyte juncitures leads to increased permeability of the glomerulus to macromolecules or
15 "leakiness" of the glomerular capsule. Proliferative effects are also observed in mesangial, epithelial and endothelial cells, as well as increases in the deposition of collagen and other matrix proteins. Sclerosis of both the glomeruli and tubules is another common symptom of the hypertrophied nephrons and the risk of coagulation in the glomerulus is increased. In particular, these adaptations of the remaining nephrons, by pushing the SNGFR well beyond its normal level,
20 actually decrease the capacity of the remaining nephrons to respond to acute changes in water, solute, or acid loads and, therefore, actually increase the probability of additional nephron loss.

As chronic renal failure progresses, and GFR continues to decline to less than 10% of normal (e.g., 5-10 ml/min), the subject enters end-stage renal disease (ESRD). During this phase, the inability of the remaining nephrons to adequately remove waste products from the blood,
25 while retaining useful products and maintaining fluid and electrolyte balance, leads to a rapid decline in which many organ systems, and particularly the cardiovascular system, may begin to fail. For example, BUN and creatinine levels may be expected to rise and, at BUN levels of 60-100 mg/dL and serum creatinine levels of 8-12 mg/dL, a uremic syndrome will typically develop in which the kidneys can no longer remove the end products of nitrogen metabolism. At this
30 point, renal failure will rapidly progress to death unless the subject receives renal replacement therapy (i.e., chronic hemodialysis, continuous peritoneal dialysis, or kidney transplantation).

- 4 -

Approximately 600 patients per million receive chronic dialysis each year in the United States, at an average cost approaching \$60,000-\$80,000 per patient per year. Of the new cases of end-stage renal disease each year, approximately 28-33% are due to diabetic nephropathy (or diabetic glomerulopathy or diabetic renal hypertrophy), 24-29% are due to hypertensive nephrosclerosis (or hypertensive glomerulosclerosis), and 15-22% are due to glomerulonephritis.

5 The 5-year survival rate for all chronic dialysis patients is approximately 40%, but for patients over 65, the rate drops to approximately 20%.

Morphogens and Growth Factors

A great many proteins have now been identified which appear to act as morphogenetic or growth factors, regulating cell proliferation or differentiation. Typically these growth factors exert their effects on specific sets or subsets of cells or tissues. Thus, for example, epidermal growth factors, nerve growth factors, fibroblast growth factors, various hormones, and many other proteins inducing or inhibiting cell proliferation or differentiation have been identified and shown to affect some subgroup of cells or tissues.

15 One group of morphogenetic proteins, referred to herein as "morphogens," includes members of the family of osteogenic proteins/bone morphogenetic proteins (OP/BMPs) which were initially identified by their ability to induce ectopic, endochondral bone morphogenesis. Subsequent characterization of the nucleic acid and amino acid sequences of the BMPs has shown them to be a subgroup of the TGF- β superfamily of growth factors. Members of this morphogen 20 family have now been shown to include the mammalian osteogenic protein-1 (OP-1, also known as BMP-7), osteogenic protein-2 (OP-2), osteogenic protein-3 (OP-3), BMP-2 (also known as BMP-2A or CBMP-2A), BMP-3, BMP-4 (also known as BMP-2B or CBMP-2B), BMP-5, BMP-6, Vgr-1, and GDF-1, as well as the Xenopus homologue Vgl and the Drosophila homologues DPP and 60A. Members of this family encode secreted polypeptides that share 25 common structural features and that are similarly processed from pro-proteins to yield carboxy terminal mature proteins having a conserved pattern of cysteines. The active forms of these proteins are either disulfide-bonded homodimers of a single family member, or heterodimers of two different members (see, e.g., Massague (1990) Annu. Rev. Cell Biol. 6:597; Sampath, et al. (1990) J. Biol. Chem. 265:13198).

30 The members of the morphogen family of proteins are expressed in a variety of tissues during development. BMP-3 for, example, has been shown to be expressed in developing human lung and kidney (Vukicevic et al. (1994) J. Histochem. Cytochem. 42:869-875), BMP-4 has been

- 5 -

shown to be expressed in the developing limbs, heart, facial processes and condensed mesenchyme associated with early whisker follicles in embryonic mice (Jones, et al. (1991) Development 111:531-542), and OP-1 (BMP-7) has been shown immunohistochemically to be associated with basement membranes in human embryos, including those of the developing lungs, 5 pancreas, skin, and convoluted tubules of kidneys (Vukicevic, et al. (1994) Biochem. Biophys. Res. Commun. 198:693-700). Some of the morphogens (e.g., OP-2 and BMP-2) were not detected in analyses of adult tissues, suggesting only an early developmental role for these morphogens (Ozkaynak, et al. (1992) J. Biol. Chem. 267:25220-25227). In contrast, high levels of murine OP-1 expression have been observed in adult mouse kidneys (Ozkaynak, et al. (1991) 10 Biochem. Biophys. Res. Commun. 179:116-123). This suggests a possible role for OP-1 synthesized in the kidney as a paracrine regulator of bone growth, and would be consistent with the role of the kidneys in both calcium regulation and bone homeostasis.

A great variety of growth factors have been considered which may participate in the regulation of the growth and repair of renal tissues (reviewed in, e.g., Toback (1992) Kidney Intl. 15 41:226-246). For example, EGF, TGF- α , TGF- β , IGF-I, IGF-II, PDGF, FGF, Renin/Angiotensin II, IL-1 and OP-1 have all been found to be expressed by various adult renal cells or tissues and to have effects on renal cell proliferation or differentiation (see, Toback (1992) supra, Ozkaynak, et al. (1991) supra). In addition, several of these have been found to be expressed in the developing kidney, including IGF-I, TGF- β and OP-1 (reviewed in, e.g., Bard, et al. (1994) Mech. Develop. 20 48:3-11).

Interestingly, TGF- β has been shown in a murine metanephric organ culture system to retard overall growth and segmental differentiation of all segments of developing nephrons except the thick ascending limb-early distal tubules (Avner and Sweeney (1990) Pediatr. Nephrol. 4:372-377). In addition, TGF- β expression has been found to be increased in several models of renal 25 disease, suggesting that TGF- β mediated increases in the synthesis of extracellular matrix components may be involved in the etiology of diabetic nephropathy (or diabetic glomerulopathy or diabetic renal hypertrophy), renal fibrosis, glomerulosclerosis and glomerulonephritis, interstitial fibrosis, and hypertensive nephrosclerosis (Shankland, et al. (1994) Kidney Intl. 46:430-442; Yamamoto, et al. (1994) Kidney Intl. 45:916-927; Yamamoto, et al. (1993) PNAS 30 90:1814-1818; Tamaki, et al. (1994) Kidney Intl. 45:525-536; Border, et al. (1990) Nature 346:371-374; Hamaguchi, et al. (1995) Hypertension 26:199-207).

Also of interest is the fact that serum levels of human growth hormone (GH) are elevated in subjects with chronic renal failure (Wright et al. (1968) Lancet 2:798; Samaan and Freeman (1970) Metabolism 19:102). Recombinant GH has been shown to help maintain protein balance in malnourished chronic renal failure patients, and to promote "catch-up" growth in children with 5 chronic renal failure. It has been suggested that these effects are mediated by IGF-I (see, e.g., Kopple (1992) Miner. Electrolyte Metab. 18:269-275). Although some studies have found that the administration of IGF-I increases renal plasma flow and GFR in chronic renal failure patients (e.g., Guler, et al. (1989) PNAS 86:2868-2872; Hirschberg, et al. (1993) Kidney Intl. 43:387-397), other studies have found that this effect is merely transient (Miller, et al. (1994) Kidney Intl. 10 46:201-207).

Thus, although some growth factors have been shown to be expressed in both developing and adult renal tissues, and although at least one has been shown to increase renal function in the short term, none has yet been shown to be of therapeutic benefit in preventing, inhibiting, or delaying the progressive loss of renal function that characterizes chronic renal failure. A need 15 remains, therefore, for treatments which will prevent the progressive loss of renal function which causes hundreds of thousand of patients to become dependent upon chronic dialysis, and which results in the premature deaths of tens of thousands each year.

Summary of the Invention

The present invention is directed to methods of treatment, and pharmaceutical 20 preparations for use in the treatment, of mammalian subjects in, or at risk of, chronic renal failure, or at risk of the need for renal replacement therapy. Such subjects include subjects already afflicted with chronic renal failure, or which have already received renal replacement therapy, as well as any subject reasonably expected to suffer a progressive loss of renal function associated with progressive loss of functioning nephron units. Whether a particular subject is at risk is a 25 determination which may routinely be made by one of ordinary skill in the relevant medical or veterinary art. Subjects in, or at risk of, chronic renal failure, or at risk of the need for renal replacement therapy, include but are not limited to the following: subjects which may be regarded as afflicted with chronic renal failure, end-stage renal disease, chronic diabetic nephropathy, hypertensive nephrosclerosis, chronic glomerulonephritis, hereditary nephritis, and/or renal 30 dysplasia; subjects having a biopsy indicating glomerular hypertrophy, tubular hypertrophy, chronic glomerulosclerosis, and/or chronic tubulointerstitial sclerosis; subjects having an

- ultrasound, MRI, CAT scan, or other non-invasive examination indicating renal fibrosis; subjects having an unusual number of broad casts present in urinary sediment; subjects having a GFR which is chronically less than about 50%, and more particularly less than about 40%, 30% or 20%, of the expected GFR for the subject; human male subjects weighing at least about 50 kg and
- 5 having a GFR which is chronically less than about 50 ml/min, and more particularly less than about 40 ml/min, 30 ml/min or 20 ml/min; human female subjects weighing at least about 40 kg and having a GFR which is chronically less than about 40 ml/min, and more particularly less than about 30 ml/min, 20 ml/min or 10 ml/min; subjects possessing a number of functional nephron units which is less than about 50%, and more particularly less than about 40%, 30% or 20%, of
- 10 the number of functional nephron units possessed by a healthy but otherwise similar subject; subjects which have a single kidney; and subjects which are kidney transplant recipients.

The methods and compositions of this invention capitalize in part upon the discovery that certain proteins of eukaryotic origin may be used as renal therapeutic agents in the treatment of subjects at risk, as defined herein, of chronic renal failure or the need for renal replacement therapy. Generally, these renal therapeutic agents are proteins, or are based upon proteins, which are members of the osteogenic protein/bone morphogenetic protein (OP/BMP) family of proteins. Thus, useful OP/BMP renal therapeutic agents of the invention include polypeptides, or functional variants of polypeptides, comprising at least the C-terminal six- or seven-cysteine domain of a mammalian protein selected from the group consisting of OP-1, OP-2, OP-3, BMP2, BMP3,

15 BMP4, BMP5, BMP6, BMP9, and proteins which exhibit at least 70% or, more preferably, 75% or 80% amino acid sequence homology with the amino acid sequence of the seven-cysteine domain of human OP-1; and which are (a) capable of inducing chondrogenesis in the Reddi-Sampath ectopic bone assay (Sampath and Reddi (1981), Proc. Natl. Acad. Sci. (USA) 78:7599-7603) or a substantially equivalent assay, (b) capable of significantly preventing, inhibiting,

20 delaying or alleviating the progressive loss of renal function in a standard animal model of chronic renal failure, or (c) capable of causing a clinically significant improvement in a standard marker of renal function when administered to a mammal in, or at risk of, chronic renal failure. More generally speaking, the invention provides for the use of "morphogens" which are dimeric proteins that induce morphogenesis of one or more eukaryotic (e.g., mammalian) cells, tissues or organs.

25 Of particular interest herein are morphogens that induce morphogenesis at least of mammalian renal tissue, including formation of functional renal epithelium and, in particular, functional glomerular and tubular epithelium. Morphogens comprise a pair of polypeptides that, when

folded, adopt a configuration sufficient for the resulting dimeric protein to elicit morphogenetic responses in cells and tissues displaying receptors specific for said morphogen. That is, morphogens generally induce all of the following biological functions in a morphogenically permissive environment: stimulating proliferation of progenitor cells; stimulating the 5 differentiation of progenitor cells; stimulating the proliferation of differentiated cells; and supporting the growth and maintenance of differentiated cells. "Progenitor" cells are uncommitted cells that are competent to differentiate into one or more specific types of differentiated cells, depending on their genomic repertoire and the tissue specificity of the permissive environment in which morphogenesis is induced. Morphogens further can delay or 10 mitigate the onset of senescence- or quiescence-associated loss of phenotype and/or tissue function. Morphogens still further can stimulate phenotypic expression of differentiated cells, including expression of metabolic and/or functional, e.g., secretory, properties thereof. In addition, morphogens can induce redifferentiation of committed cells under appropriate environmental conditions. As noted above, morphogens that induce proliferation and/or 15 differentiation at least of mammalian renal tissue, and/or support the growth, maintenance and/or functional properties of mammalian nephrons, are of particular interest herein.

In preferred embodiments, the pair of morphogen polypeptides have amino acid sequences each comprising a sequence that shares a defined relationship with an amino acid sequence of a reference morphogen. Herein, preferred morphogen polypeptides share a defined relationship 20 with a sequence present in morphogenically active human OP-1, SEQ ID NO: 4. However, any one or more of the naturally occurring or biosynthetic sequences disclosed herein similarly could be used as a reference sequence. Preferred morphogen polypeptides share a defined relationship with at least the C-terminal six cysteine domain of human OP-1, residues 43-139 of SEQ ID NO: 4. Preferably, morphogen polypeptides share a defined relationship with at least the C- 25 terminal seven cysteine domain of human OP-1, residues 38-139 of SEQ ID NO: 4. That is, preferred morphogen polypeptides in a dimeric protein with morphogenic activity each comprise a sequence that corresponds to a reference sequence or is functionally equivalent thereto.

Functionally equivalent sequences include functionally equivalent arrangements of cysteine residues disposed within the reference sequence, including amino acid insertions or deletions 30 which alter the linear arrangement of these cysteines, but do not materially impair their relationship in the folded structure of the dimeric morphogen protein, including their ability to form such intra- or inter-chain disulfide bonds as may be necessary for morphogenic activity.

Functionally equivalent sequences further include those wherein one or more amino acid residues differs from the corresponding residue of a reference morphogen sequence, e.g., the C-terminal seven cysteine domain (or "skeleton") of human OP-1, provided that this difference does not destroy morphogenic activity. Accordingly, conservative substitutions of corresponding amino acids in the reference sequence are preferred. Amino acid residues that are "conservative substitutions" for corresponding residues in a reference sequence are those that are physically or functionally similar to the corresponding reference residues, e.g., that have similar size, shape, electric charge, chemical properties including the ability to form covalent or hydrogen bonds, or the like. Particularly preferred conservative substitutions are those fulfilling the criteria defined for an "accepted point mutation" in Dayhoff et al. (1978), 5 Atlas of Protein Sequence and Structure, Suppl. 3, ch. 22 (pp. 354-352), Natl. Biomed. Res. Found., Washington, D.C. 20007, the teachings of which are incorporated by reference herein.

In certain embodiments, a polypeptide suspected of being functionally equivalent to a reference morphogen polypeptide is aligned therewith using the method of Needleman, et al. (1970), J. Mol. Biol. 48:443-453, implemented conveniently by computer programs such as the Align program (DNAStar, Inc.). As noted above, internal gaps and amino acid insertions in the candidate sequence are ignored for purposes of calculating the defined relationship, conventionally expressed as a level of amino acid sequence homology or identity, between the candidate and reference sequences. "Amino acid sequence homology" is understood herein to mean amino acid sequence similarity. Homologous sequences share identical or similar amino acid residues, where similar residues are conservative substitutions for, or "allowed point mutations" of, corresponding amino acid residues in an aligned reference sequence. Thus, a candidate polypeptide sequence that shares 70% amino acid homology with a reference sequence is one in which any 70% of the aligned residues are either identical to or are conservative substitutions of the corresponding residues in a reference sequence.

Of particular interest herein are morphogens, which, when provided to the kidney of a mammal, induce or maintain the normal state of differentiation and growth of nephron units. Of still more particular interest herein are morphogens which, when administered to a mammal, prevent, inhibit or delay the development of compensatory hypertrophy, including glomerular hypertrophy and/or tubular hypertrophy. Such morphogens can be used to treat a mammal in, or at risk of, chronic renal failure by preventing, inhibiting or delaying the progressive loss of functional nephron units and the consequent progressive loss of renal function.

The present invention alternatively can be practiced with methods and compositions comprising a morphogen stimulating agent or morphogen inducer in lieu of a morphogen. A "morphogen inducer" is a compound that stimulates in vivo production, e.g., expression, of a therapeutically effective concentration of an endogenous morphogen in the body of a mammal 5 sufficient to regenerate or maintain renal tissue and/or to inhibit additional loss thereof. Such compounds are understood to include substances which, when administered to a mammal, act on cells of tissue(s) or organ(s) that normally are competent to produce and/or secrete a morphogen encoded within the genome of the mammal, and which cause the endogenous level of the morphogen in the mammal's body to be altered. Endogenous or administered morphogens can 10 act as endocrine, paracrine or autocrine factors. That is, endogenous morphogens can be synthesized by the cells in which morphogenetic responses are induced, by neighboring cells, or by cells of a distant tissue, in which circumstances the secreted endogenous morphogen is transported to the site of morphogenesis, e.g., by the individual's bloodstream. In preferred embodiments, the agent stimulates expression and/or secretion of an endogenous morphogen so 15 as to increase amounts thereof in renal tissues.

In still other embodiments, an agent which acts as an agonist of a morphogen receptor may be administered instead of the morphogen itself. An "agonist" of a receptor means a compound which binds to the receptor and for which such binding has a similar functional result as binding of the natural, endogenous ligand of the receptor. That is, the compound must, upon 20 interaction with the receptor, produce the same or substantially similar transmembrane and/or intracellular effects as the endogenous ligand. Thus, an agonist of a morphogen receptor binds to the receptor and such binding has the same or a similar functional result as morphogen binding (e.g., induction of morphogenesis). The activity or potency of an agonist can be less than that of the natural ligand, in which case the agonist is said to be a "partial agonist," or it can be equal to 25 or greater than that of the natural ligand, in which case it is said to be a "full agonist." Thus, for example, a small peptide or other molecule which can mimic the activity of a morphogen in binding to and activating the morphogen's receptor may be employed as an equivalent of the morphogen. Preferably the agonist is a full agonist, but partial morphogen receptor agonists may also be advantageously employed. Methods of identifying such agonists are known in the art and 30 include assays for compounds which induce morphogen-mediated responses (e.g., induction of differentiation of metanephric mesenchyme, induction of endochondral bone formation, and the like). Such an agent may also be referred to as a morphogen "mimic," "mimetic," or "analog."

- 11 -

The OP/BMP renal therapeutic agents of the invention, or the morphogens, morphogen inducers and agonists of morphogen receptors of the invention, may be administered by any route of administration which is compatible with the selected agent, and may be formulated with any pharmaceutically acceptable carrier appropriate to the route of administration. Preferred routes of 5 administration are parenteral and, in particular, intravenous, intraperitoneal, and renal intracapsular. Treatments are also preferably conducted over an extended period on an outpatient basis. Daily dosages of the renal therapeutic agents are expected to be in the range of about 0.01-1000 µg/kg body weight, and more preferably about 10-300 µg/kg body weight, although precise 10 dosages will vary depending upon the particular renal therapeutic agent employed and the particular subject's medical condition and history.

Finally, in yet further embodiments, renal cells may be implanted into the kidney of a subject in, or at risk, chronic renal failure, or at risk of needing renal replacement therapy, in order to serve as a source of morphogen and/or to provide a source of additional functional renal tissue. These cells may be renal mesenchymal progenitor cells, or renal mesenchymal progenitor cells 15 which have been induced to undergo metanephric differentiation. The cells may be derived from a donor (e.g., a tissue-type matched donor, sibling, identical twin), may be derived from a tissue culture (e.g., undifferentiated renal mesenchyme culture, fetal renal tissue culture), or may be explanted from the subject and then be re-implanted after proliferation and/or differentiation. Preferably, the cells are induced to undergo metanephric differentiation by treatment with a 20 morphogen (e.g., OP-1) either before or after implantation.

The treatments of the present invention are useful in preventing, inhibiting or delaying the progressive loss of functional nephron units, and the consequent progressive loss of renal function, which typify chronic renal failure. As such they are of great value in preventing or delaying the need for chronic dialysis or renal replacement therapy in subjects with chronic renal 25 insufficiency, or reducing the necessary frequency of chronic renal dialysis in subjects with end-stage renal disease. As such, they are useful in prolonging the lives, and in maintaining the quality of life, of subjects at risk of, or already afflicted with, chronic renal failure.

Brief Description of the Figures

Figure 1. This figure is a bar graph showing average serum creatinine levels for groups of 30 sham-operated ("SHAM") or partially nephrectomized ("Nx Contr" and "OP-1") rats. 5-6 months

- 12 -

post-surgery, rats received injections of vehicle only ("Nx control" and "SHAM") or 1, 3, 10 or 50 µg/kg body weight of soluble OP-1 ("OP-1") three times a week for 4-8 weeks.

Figure 2. This figure is a bar graph showing average serum urea levels for groups of sham-operated ("SHAM") or partially nephrectomized ("Nx Contr" and "OP-1") rats. 5-6 months post-surgery, rats received injections of vehicle only ("Nx control" and "SHAM") or 1, 3, 10 or 50 µg/kg body weight of soluble OP-1 ("OP-1") three times a week for 4-8 weeks.

Figure 3. Panels A-C of this figure are micrographs of renal tissue from rats at 10x magnification. (A) Tissue from sham-operated rat. (B) Tissue from rat in chronic renal failure after 5/6 nephrectomy (Nx control). (C) Tissue from rat treated with OP-1 after 5/6 nephrectomy.

Figure 4. Panels A-C of this figure are micrographs of renal tissue from rats at 40x magnification. (A) Tissue from sham-operated rat. (B) Tissue from rat in chronic renal failure after 5/6 nephrectomy (Nx control). (C) Tissue from rat treated with OP-1 after 5/6 nephrectomy.

Figure 5. This figure is a line graph showing average serum creatinine levels over 9 weeks for groups of partially nephrectomized rats. 2-3 weeks post-surgery, rats received injections of vehicle only ("Control") or 10 µg/kg body weight of soluble OP-1 ("OP-1") 3 times per week.

Figure 6. This figure is a line graph showing average creatinine clearance rates as a measure of GFR over 8 weeks for groups of partially nephrectomized rats. 2-3 weeks post-surgery, rats received injections of vehicle only ("Control") or 10 µg/kg body weight of soluble OP-1 ("OP-1") 3 times per week.

Figure 7. Panels 7-1 through 7-12 of this figure are a tabular alignment of the amino acid sequences of various naturally occurring morphogens with a preferred reference sequence of human OP-1, residues 38-139 of SEQ ID NO: 4. Morphogen polypeptides shown in this figure also are identified in the Sequence Listing.

Detailed Description of the Invention**I. Definitions**

In order to more clearly and concisely point out the subject matter of the claimed invention, the following definitions are provided for specific terms used in the following written 5 description and appended claims.

Renal therapeutic agent. As used herein, the term "renal therapeutic agent" means a polypeptide, or a functional variant of a polypeptide, comprising at least the C-terminal six- or seven-cysteine domain of a mammalian protein selected from the group consisting of OP-1, OP-2, OP-3, BMP2, BMP3, BMP4, BMP5, BMP6, BMP9, and proteins which exhibit at least 70% or, 10 more preferably, 75% or 80% amino acid sequence homology with the amino acid sequence of the seven-cysteine domain of human OP-1; and which is (a) capable of inducing chondrogenesis in the Reddi-Sampath ectopic bone assay (Sampath and Reddi (1981), Proc. Natl. Acad. Sci. (USA) 78:7599-7603) or a substantially equivalent assay, (b) capable of significantly preventing, inhibiting, delaying or alleviating the progressive loss of renal function in a standard animal model 15 of chronic renal failure, or (c) capable of causing a clinically significant improvement in a standard marker of renal function when administered to a mammal in, or at risk of, chronic renal failure. As used herein, a percentage "homology" between two amino acid sequences indicates the percentage of amino acid residues which are identical or similar between the sequences and, as used herein, "similar" residues are "conservative substitutions" which fulfill the criteria defined for 20 an "accepted point mutation" in Dayhoff et al. (1978), Atlas of Protein Sequence and Structure Vol. 5 (Suppl. 3), pp. 354-352, Natl. Biomed. Res. Found., Washington, D.C.

Therapeutic efficacy. As used herein, a renal therapeutic agent of the invention is said to have "therapeutic efficacy," and an amount of the agent is said to be "therapeutically effective," if administration of that amount of the agent is sufficient to cause a clinically significant 25 improvement in a standard marker of renal function when administered to a mammalian subject (e.g., a human patient) in, or at risk of, chronic renal failure. Such markers of renal function are well known in the medical literature and include, without being limited to, rates of increase in BUN levels, rates of increase in serum creatinine, static measurements of BUN, static measurements of serum creatinine, glomerular filtration rates (GFR), ratios of BUN/creatinine, 30 serum concentrations of sodium (Na⁺), urine/plasma ratios for creatinine, urine/plasma ratios for urea, urine osmolality, daily urine output, and the like (see, for example, Brenner and Lazarus (1994), in Harrison's Principles of Internal Medicine, 13th edition, Isselbacher et al., eds.,

- 14 -

McGraw Hill Text, New York; Luke and Strom (1994), in Internal Medicine, 4th Edition, J.H. Stein, ed., Mosby-Year Book, Inc. St. Louis.)

Glomerular Filtration Rate (GFR). The "glomerular filtration rate" or "GFR" is proportional to the rate of clearance into urine of a plasma-borne substance which is not bound by serum proteins, is freely filtered across glomeruli, and is neither secreted nor reabsorbed by the renal tubules. Thus, as used herein, GFR preferably is defined by the following equation:

$$GFR = \frac{U_{conc} \times V}{P_{conc}}$$

where U_{conc} is the urine concentration of the marker, P_{conc} is the plasma concentration of the marker, and V is the urine flow rate in ml/min. Optionally, GFR is corrected for body surface area. Thus, the GFR values used herein may be regarded as being in units of ml/min/1.73m².

The preferred measure of GFR is the clearance of inulin but, because of the difficulty of measuring the concentrations of this substance, the clearance of creatinine is typically used in clinical settings. For example, for an average size, healthy human male (70 kg, 20-40 yrs), a typical GFR measured by creatinine clearance is expected to be approximately 125 ml/min with plasma concentrations of creatinine of 0.7-1.5 mg/dL. For a comparable, average size woman, a typical GFR measured by creatinine clearance is expected to be approximately 115 ml/min with creatinine levels of 0.5-1.3 mg/dL. During times of good health, human GFR values are relatively stable until about age 40, when GFR typically begins to decrease with age. For subjects surviving to age 85 or 90, GFR may be reduced to 50% of the comparable values at age 40.

Expected Glomerular Filtration Rate (GFR_{exp}). An estimate of the "expected GFR" or "GFR_{exp}" may be provided based upon considerations of a subject's age, weight, sex, body surface area, and degree of musculature, and the plasma concentration of some marker compound (e.g., creatinine) as determined by a blood test. Thus, as an example, an expected GFR or GFR_{exp} may be estimated as:

$$25 \quad GFR_{exp} \approx \frac{(140 - age) \times weight(kg)}{72 \times P_{conc} (mg/dL)}$$

This estimate does not take into consideration such factors as surface area, degree of musculature, or percentage body fat. Nonetheless, using plasma creatinine levels as the marker, this formula has been employed for human males as an inexpensive means of estimating GFR. Because creatinine is produced by striated muscle, the expected GFR or GFR_{exp} of human female subjects

is estimated by the same equation multiplied by 0.85 to account for expected differences in muscle mass. (See Lemann, et al. (1990) Am. J. Kidney Dis. 16(3):236-243.)

Broad Cast. Microscopic examination of urinary sediment for the presence of formed elements is a standard procedure in urinalysis. Amongst the formed elements which may be present in urine are cylindrical masses of agglutinated materials that typically represent a mold or "cast" of the lumen of a distal convoluted tubule or collecting tubule. In healthy human subjects, such casts typically have a diameter of 15-25 μm . In subjects with chronic renal failure, however, hypertrophy of the tubules may result in the presence of "broad casts" or "renal failure casts" which are 2-6 times the diameter of normal casts and often have a homogeneous waxy appearance. Thus, as used herein, a "broad cast" means a urinary sediment cast having a diameter of 2-6 times normal, or about 30-150 μm for human casts.

Chronic. As used herein with respect to clinical indications such as urinary casts, measured GFR, or other markers of renal function, "chronic" means persisting for a period of at least three, and more preferably, at least six months. Thus, for example, a subject with a measured GFR chronically below 50% of GFR_{exp} is a subject in which the GFR has been measured and found to be below 50% of GFR_{exp} in at least two measurements separated by at least three, and more preferably, by at least six months, and for which there is no medically sound reason to believe that GFR was substantially (e.g., 10%) higher during the intervening period.

Subjects in, or at risk of, chronic renal failure. As used herein, a subject is said to be in, or at risk of, chronic renal failure, or at risk of the need for renal replacement therapy, if the subject is reasonably expected to suffer a progressive loss of renal function associated with progressive loss of functioning nephron units. Whether a particular subject is in, or at risk of, chronic renal failure is a determination which may routinely be made by one of ordinary skill in the relevant medical or veterinary art. Subjects in, or at risk of, chronic renal failure, or at risk of the need for renal replacement therapy, include but are not limited to the following: subjects which may be regarded as afflicted with chronic renal failure, end-stage renal disease, chronic diabetic nephropathy, hypertensive nephrosclerosis, chronic glomerulonephritis, hereditary nephritis, and/or renal dysplasia; subjects having a biopsy indicating glomerular hypertrophy, tubular hypertrophy, chronic glomerulosclerosis, and/or chronic tubulointerstitial sclerosis; subjects having an ultrasound, MRI, CAT scan, or other non-invasive examination indicating renal fibrosis; subjects having an unusual number of broad casts present in urinary sediment; subjects having a GFR which is chronically less than about 50%, and more particularly less than about 40%, 30% or

20%, of the expected GFR for the subject; human male subjects weighing at least about 50 kg and having a GFR which is chronically less than about 50 ml/min, and more particularly less than about 40 ml/min, 30 ml/min or 20 ml/min; human female subjects weighing at least about 40 kg and having a GFR which is chronically less than about 40 ml/min, and more particularly less than about 30 ml/min, 20 ml/min or 10 ml/min; subjects possessing a number of functional nephron units which is less than about 50%, and more particularly less than about 40%, 30% or 20%, of the number of functional nephron units possessed by a healthy but otherwise similar subject; subjects which have a single kidney; and subjects which are kidney transplant recipients.

II. Description of the Preferred Embodiments

10 A. General

The present invention depends, in part, upon the surprising discovery that administration of certain protein-based renal therapeutic agents to subjects in, or at risk of, chronic renal failure, can reduce mortality and/or morbidity rates, and prevent, inhibit, delay or alleviate the progressive loss of renal function which characterizes chronic renal failure. Alternatively, or in addition, 15 administration of the renal therapeutic agents of the present invention can prevent, inhibit or delay the progressive loss of functional nephron units and the progressive decline in glomerular filtration rate (GFR) which slowly but inevitably leads to the need for renal replacement therapy (i.e., renal transplant or chronic dialysis) or death. In preferred embodiments, the therapeutic agents of the invention are members of the osteogenic protein/bone morphogenetic protein (OP/BMP) family 20 within the TGF- β superfamily of proteins.

B. Renal Therapeutic Agents

The renal therapeutic agents of the present invention are naturally occurring proteins, or functional variants of naturally occurring proteins, in the osteogenic protein/bone morphogenetic protein (OP/BMP) family within the TGF- β superfamily of proteins. That is, these proteins form 25 a distinct subgroup, referred to herein as the "OP/BMP family," within the loose evolutionary grouping of sequence-related proteins known as the TGF- β superfamily. Members of this protein family comprise secreted polypeptides that share common structural features, and that are similarly processed from a pro-protein to yield a carboxy-terminal mature protein. Within the mature protein, all members share a conserved pattern of six or seven cysteine residues defining a 30 97-106 amino acid domain, and the active form of these proteins is either a disulfide-bonded homodimer of a single family member, or a heterodimer of two different members (see, e.g., Massague (1990), Annu. Rev. Cell Biol. 6:597; Sampath et al. (1990), J. Biol. Chem.

- 17 -

265:13198). For example, in its mature, native form, natural-sourced human OP-1 is a glycosylated dimer typically having an apparent molecular weight of about 30-36 kDa as determined by SDS-PAGE. When reduced, the 30 kDa protein gives rise to two glycosylated peptide subunits having apparent molecular weights of about 16 kDa and 18 kDa. The 5 unglycosylated protein has an apparent molecular weight of about 27 kDa. When reduced, the 27 kDa protein gives rise to two unglycosylated polypeptide chains, having molecular weights of about 14 kDa to 16 kDa.

Typically, the naturally occurring OP/BMP proteins are translated as a precursor, having an N-terminal signal peptide sequence, a "pro" domain, and a "mature" protein domain. The 10 signal peptide is typically less than 30 residues, and is cleaved rapidly upon translation at a cleavage site that can be predicted using the method of Von Heijne (1986), Nucleic Acids Research 14:4683-4691. The "pro" domain is variable both in sequence and in length, ranging from approximately 200 to over 400 residues. The pro domain is cleaved to yield the "mature" C-terminal domain of approximately 115-180 residues, which includes the conserved six- or 15 seven-cysteine C-terminal domain of 97-106 residues. As used herein, the "pro form" of an OP/BMP family member refers to a protein comprising a folded pair of polypeptides, each comprising a pro domain in either covalent or noncovalent association with the mature domains of the OP/BMP polypeptide. Typically, the pro form of the protein is more soluble than the mature form under physiological conditions. The pro form appears to be the primary form secreted from 20 cultured mammalian cells. The "mature form" of the protein refers to mature C-terminal domain which is not associated, either covalently or noncovalently, with the pro domain. Any preparation of OP-1 is considered to contain mature form when the amount of pro domain in the preparation is no more than 5% of the amount of "mature" C-terminal domain.

OP/BMP family members useful herein include any of the known naturally-occurring 25 native proteins including allelic, phylogenetic counterpart and other variants thereof, whether naturally-sourced or biosynthetically produced (e.g., including "muteins" or "mutant proteins"), as well as new, active members of the OP/BMP family of proteins.

Particularly useful sequences include those comprising the C-terminal seven cysteine domains of mammalian, preferably human, human OP-1, OP-2, OP-3, BMP2, BMP3, BMP4, 30 BMP5, BMP6, BMP8 and BMP9. Other proteins useful in the practice of the invention include active forms of GDF-5, GDF-6, GDF-7, DPP, Vg1, Vgr-1, 60A, GDF-1, GDF-3, GDF-5, GDF-6, GDF-7, BMP10, BMP11, BMP13, BMP15, UNIVIN, NODAL, SCREW, ADMP or

- 18 -

NURAL and amino acid sequence variants thereof. In one currently preferred embodiment, the renal therapeutic agents of the invention are selected from any one of: OP-1, OP-2, OP-3, BMP2, BMP3, BMP4, BMP5, BMP6, and BMP9.

Publications disclosing these sequences, as well as their chemical and physical properties, 5 include: OP-1 and OP-2: U.S. Pat. No. 5,011,691, U.S. Pat. No. 5,266,683, and Ozkaynak et al. (1990), EMBO J. 9:2085-2093; OP-3: WO94/10203; BMP2, BMP3, and BMP4: U.S. Pat. No. 5,013,649, WO91/18098, WO88/00205, and Wozney et al. (1988), Science 242:1528-1534; BMP5 and BMP6: WO90/11366 and Celeste et al. (1991), Proc. Natl. Acad. Sci. (USA) 87:9843-9847; Vgr-1: Lyons et al. (1989), Proc. Natl. Acad. Sci. (USA) 86: 4554-4558; DPP: 10 Padgett et al. (1987), Nature 325:81-84; Vgl: Weeks (1987), Cell 51:861-867; BMP-9: WO95/33830; BMP10: WO94/26893; BMP-11: WO94/26892; BMP12: WO95/16035; BMP-13: WO95/16035; GDF-1: WO92/00382 and Lee et al. (1991), Proc. Natl. Acad. Sci. (USA) 88:4250-4254; GDF-8: WO94/21681; GDF-9: WO94/15966; GDF-10: WO95/10539; 15 GDF-11: WO96/01845; BMP-15: WO96/36710; MP121: WO96/01316; GDF-5 (CDMP-1, MP52): WO94/15949, WO96/14335, WO93/16099 and Storm et al. (1994), Nature 368:639-643; GDF-6 (CDMP-2, BMP13): WO95/01801, WO96/14335 and WO95/10635; GDF-7 (CDMP-3, BMP12): WO95/10802 and WO95/10635; BMP-3b: Takao, et al. (1996), Biochem. Biophys. Res. Comm. 219:656-662; GDF-3: WO94/15965; 60A: Blaster et al. (1993), Cell 73:687-702 and GenBank accession number L12032. In another embodiment, useful proteins 20 include biologically active biosynthetic constructs, including novel biosynthetic proteins and chimeric proteins designed using sequences from two or more known OP/BMP family proteins. See also the biosynthetic constructs disclosed in U.S. Pat. No. 5,011,691, the disclosure of which is incorporated herein by reference (e.g., COP-1, COP-3, COP-4, COP-5, COP-7, and COP-16).

In other preferred embodiments, the renal therapeutic agents useful herein include 25 therapeutically effective proteins in which the amino acid sequences comprise a sequence sharing at least 70% amino acid sequence "homology" and, preferably, 75% or 80% homology with the C-terminal seven cysteine domain present in the active forms of human OP-1 (i.e., residues 330-431, as shown in SEQ ID NO: 2 of U.S. Pat. No. 5,266,683). In other preferred 30 embodiments, the renal therapeutic agents useful herein include therapeutically effective proteins in which the amino acid sequences comprise a sequence sharing at least 60% amino acid sequence identity and, preferably, 65% or 70% identity with the C-terminal seven cysteine domain present in the active forms of human OP-1. Thus, a candidate amino acid sequence thought to have

- 19 -

- therapeutic efficacy in the present invention can be aligned with the amino acid sequence of the C-terminal seven cysteine domain of human OP-1 using the method of Needleman et al. (1970), J. Mol. Biol. 48:443-453, implemented conveniently by computer programs such as the Align program (DNAstar, Inc.). As will be understood by those skilled in the art, homologous or 5 functionally equivalent sequences include functionally equivalent arrangements of the cysteine residues within the conserved cysteine domain, including amino acid insertions or deletions which alter the linear arrangement of these cysteines, but do not materially impair their relationship in the folded structure of the dimeric protein, including their ability to form such intra- or inter-chain disulfide bonds as may be necessary for biological activity. Therefore, internal gaps and amino 10 acid insertions in the candidate sequence are ignored for purposes of calculating the level of amino acid sequence homology or identity between the candidate and reference sequences.

"Amino acid sequence homology" is understood herein to include both amino acid sequence identity and similarity. Thus, as used herein, a percentage "homology" between two amino acid sequences indicates the percentage of amino acid residues which are identical or 15 similar between the sequences. "Similar" residues are "conservative substitutions" which fulfill the criteria defined for an "accepted point mutation" in Dayhoff et al. (1978), Atlas of Protein Sequence and Structure Vol. 5 (Suppl. 3), pp. 354-352, Natl. Biomed. Res. Found., Washington, D.C. Thus, "conservative substitutions" are residues that are physically or functionally similar to the corresponding reference residues, having similar size, shape, electric charge, and/or chemical 20 properties such as the ability to form covalent or hydrogen bonds, or the like. Examples of conservative substitutions include the substitution of one amino acid for another with similar characteristics, e.g., substitutions within the following groups: (a) valine, glycine; (b) glycine, alanine; (c) valine, isoleucine, leucine; (d) aspartic acid, glutamic acid; (e) asparagine, glutamine; (f) serine, threonine; (g) lysine, arginine, methionine; and (h) phenylalanine, tyrosine. The term 25 "conservative substitution" or "conservative variation" also includes the use of a substituted amino acid in place of an unsubstituted parent amino acid in a given polypeptide chain, provided that the resulting substituted polypeptide chain also has therapeutic efficacy in the present invention.

The renal therapeutic agents of the invention are also characterized by biological activities 30 which may be readily ascertained by those of ordinary skill in the art. Specifically, a renal therapeutic agent of the present invention is (a) capable of inducing chondrogenesis in the Reddi-Sampath ectopic bone assay (Sampath and Reddi (1981), Proc. Natl. Acad. Sci. (USA)

- 20 -

78:7599-7603) or a substantially equivalent assay, (b) capable of significantly preventing, inhibiting, delaying or alleviating the progressive loss of renal function in a standard animal model of chronic renal failure, or (c) capable of causing a clinically significant improvement in a standard marker of renal function when administered to a mammal in, or at risk of, chronic renal
5 failure.

The Reddi-Sampath ectopic bone assay is well known in the art as an assay of chondrogenic activity. The assay, which can be easily performed, is described and discussed in, for example, Sampath and Reddi (1981), Proc. Natl. Acad. Sci. (USA) 78:7599-7603; and Wozney (1989), "Bone Morphogenetic Proteins," Progress in Growth Factor Research 1:267-
10 280. Many equivalent assays, using other animals and tissue sites, may be employed or developed by those of skill in the art to evaluate the biological activity of the renal therapeutic agents of the present invention. See, for example, the bioassays described in U.S. Pat. No. 5,226,683.

The renal therapeutic agents of the present invention also may be tested in animal models of chronic renal failure. Mammalian models of chronic renal failure in, for example, mice, rats,
15 guinea pigs, cats, dogs, sheep, goats, pigs, cows, horses, and non-human primates, may be created by causing an appropriate direct or indirect injury or insult to the renal tissues of the animal. Animal models of chronic renal failure may, for example, be created by performing a partial (e.g., 5/6) nephrectomy which reduces the number of functioning nephron units to a level which initiates compensatory renal hypertrophy, further nephron loss, and the progressive decline in renal
20 function which characterizes chronic renal failure.

Finally, the renal therapeutic agents of the present invention may be evaluated for their therapeutic efficacy in causing a clinically significant improvement in a standard marker of renal function when administered to a mammalian subject (e.g., a human patient) in, or at risk of, chronic renal failure. Such markers of renal function are well known in the medical literature and include, without being limited to, rates of increase in BUN levels, rates of increase in serum creatinine, static measurements of BUN, static measurements of serum creatinine, glomerular filtration rates (GFR), ratios of BUN/creatinine, serum concentrations of sodium (Na⁺), urine/plasma ratios for creatinine, urine/plasma ratios for urea, urine osmolality, daily urine output, and the like (see, for example, Brenner and Lazarus (1994), in Harrison's Principles of Internal Medicine, 13th edition, Isselbacher et al., eds., McGraw Hill Text, New York; Luke and Strom (1994), in Internal Medicine, 4th Edition, J.H. Stein, ed., Mosby-Year Book, Inc. St. Louis.)

- 21 -

The renal therapeutic agents contemplated herein can be expressed from intact or truncated genomic or cDNA or from synthetic DNAs in prokaryotic or eukaryotic host cells. The dimeric proteins can be isolated from the culture media and/or refolded and dimerized in vitro to form biologically active compositions. Heterodimers can be formed in vitro by combining 5 separate, distinct polypeptide chains. Alternatively, heterodimers can be formed in a single cell by co-expressing nucleic acids encoding separate, distinct polypeptide chains. See, for example, WO93/09229, or U.S. Pat. No. 5,411,941, for several exemplary recombinant heterodimer protein production protocols. Currently preferred host cells include, without limitation, prokaryotes including E. coli, or eukaryotes including yeast, Saccharomyces, insect cells, or 10 mammalian cells, such as CHO, COS or BSC cells. One of ordinary skill in the art will appreciate that other host cells can be used to advantage. Detailed descriptions of the proteins useful in the practice of this invention, including how to make, use and test them for chondrogenic activity, are disclosed in numerous publications, including U.S. Pat. Nos. 5,266,683 and 5,011,691, the disclosures of which are herein incorporated by reference.

15 C. Morphogens, Inducers and Agonists

Table 1, below, summarizes various naturally occurring members of the OP/BMP family identified to date, including their nomenclature as used herein, their Sequence Listing references, and publication sources for the amino acid sequences for the full length proteins not included in the Sequence Listing. Each of the generic terms set forth in Table 1 is intended and should be 20 understood to embrace the therapeutically effective proteins expressed from nucleic acids encoding the identified sequence mentioned below and set forth in the Sequence Listing, or an active fragment or precursor thereof, or a functional equivalent thereof such as a naturally occurring or biosynthetic variant. Naturally occurring variants include allelic variant forms isolated from other individuals of a single biological species, as well as species variants 25 (homologues) isolated from phylogenetically distinct biological species.

TABLE 1

"OP-1" Refers generically to mammalian proteins equivalent to the human OP-1 protein disclosed in SEQ ID NO: 4 ("hOP-1"), and includes at least mouse OP-1, SEQ ID NO: 5 ("mOP-1"). In each of human and mouse OP-1, SEQ ID NOs: 4 and 5, the 30 conserved C-terminal seven cysteine domain is defined by residues 38 to 139. cDNA sequences and corresponding amino acid sequences for the full length

- 22 -

proteins are provided in SEQ ID NOs: 15 and 16 (hOP-1) and SEQ ID NOs: 17 and 18 (mOP-1.) The mature proteins are defined by residues 293-431 (hOP-1) and 292-430 (mOP-1). The "pro" regions of the proteins, cleaved to yield the mature proteins are defined essentially by residues 30-292 (hOP-1) and residues 5 30-291 (mOP-1).

- "OP-2" Refers generically to mammalian proteins equivalent to the human OP-2 protein disclosed in SEQ ID NO: 6 ("hOP-2"), and includes at least mouse OP-2 ("mOP-2", SEQ ID NO: 7). In each of human and mouse OP-2, the conserved C-terminal seven domain is defined by residues 38 to 139 of SEQ ID NOs: 6 and 7. cDNA sequences and corresponding amino acid sequences for the full length proteins are provided in SEQ ID NOs: 19 and 20 (hOP-2) and SEQ ID NOs: 21 and 22 (mOP-2.) The mature proteins are defined essentially by residues 264-402 (hOP-2) and 261-399 (mOP-2). The "pro" regions of the proteins, cleaved to yield the mature proteins are defined essentially by residues 18-263 (hOP-2) and residues 18-260 (mOP-1).
- "OP-3" Refers generically to mammalian proteins equivalent to the mouse OP-3 protein disclosed in SEQ ID NO: 26 ("mOP-3"). The conserved C-terminal seven domain is defined by residues 298 to 399 of SEQ ID NO: 26, which shares greater than 79% amino acid identity with the corresponding mOP-2 and hOP-2 sequences, and greater than 66% identity with the corresponding OP-1 sequences. A cDNA sequence encoding the above-mentioned amino acid sequence is provided in SEQ ID NO: 25. OP-3 is unique among the morphogens identified to date in that the residue at position 9 in the conserved C-terminal seven domain (e.g., residue 315 10 20 25 of SEQ ID NO: 26) is a serine, whereas other morphogens typically have a tryptophan at this location.
- "BMP-2" Refers generically to mammalian proteins equivalent to the BMP-2 proteins, including at least human BMP-2 (or CBMP-2A, SEQ ID NO: 8). The amino acid sequence for the full length proteins, referred to in the literature as BMP-2 or BMP-2A, appear in Wozney, et al. (1988) Science 242:1528-1534. The pro

- 23 -

domain for BMP-2 (BMP-2A) likely includes residues 25-248; the mature protein, residues 249-396.

"BMP-4" Refers generically to mammalian proteins equivalent to the CBMP-4 proteins, including at least human BMP-4 (or BMP-2B, SEQ ID NO: 9). The amino acid sequence for the full length proteins, referred to in the literature as BMP-4 and BMP-2B, appear in Wozney, et al. (1988) Science 242:1528-1534. The pro domain for BMP-4 (BMP-2B) likely includes residues 25-256; the mature protein, residues 257-408.

5 "DPP" refers to proteins encoded by a Drosophila DPP gene and defining a conserved C-terminal seven domain (SEQ ID NO: 10). The amino acid sequence for the full length protein appears in Padgett, et al. (1987) Nature 325:81-84. The pro domain likely extends from the signal peptide cleavage site to residue 456; the mature protein likely is defined by residues 457-588.

10 "Vgl" refers to proteins encoded by a Xenopus Vgl gene and defining a conserved C-terminal seven domain (SEQ ID NO: 11). The amino acid sequence for the full length protein appears in Weeks (1987) Cell 51:861-867. The prodomain likely extends from the signal peptide cleavage site to residue 246; the mature protein likely is defined by residues 247-360.

15 "Vgr-1" refers to proteins encoded by a murine Vgr-1 gene and defining a conserved C-terminal seven domain (SEQ ID NO: 12). The amino acid sequence for the full length protein appears in Lyons, et al. (1989) PNAS 86:4554-4558. The prodomain likely extends from the signal peptide cleavage site to residue 299; the mature protein likely is defined by residues 300-438.

20 "GDF-1" refers to proteins encoded by a human GDF-1 gene and defining a conserved C-terminal seven domain (SEQ ID NO: 13). The cDNA and encoded amino sequence for the full length protein are provided in SEQ ID NOS: 30 and 31. The prodomain likely extends from the signal peptide cleavage site to residue 214; the mature protein likely is defined by residues 215-372.

- 24 -

- "60A" refers generically to proteins expressed from a nucleic acid (e.g., the Drosophila 60A gene) encoding a 60A protein or active fragments thereof (see SEQ ID NOs: 23 and 24 wherein the cDNA and encoded amino acid sequence for the full length protein are provided). The conserved C-terminal seven domain is defined by residues 354 to 455 of SEQ ID NO: 24. The prodomain likely extends from the signal peptide cleavage site to residue 324; the mature protein likely is defined by residues 325-455. The 60A protein is considered likely to be a phylogenetic counterpart of the human and mouse OP-1 genes; Sampath, et al. (1993) PNAS 90:6004-6008.
- 5
- 10 "BMP-3" refers to proteins encoded by a human BMP-3 gene and defining a conserved C-terminal seven domain (SEQ ID NO: 26). The amino acid sequence for the full length protein appears in Wozney, et al. (1988) Science 242:1528-1534. The pro domain likely extends from the signal peptide cleavage site to residue 290; the mature protein likely is defined by residues 291-472.
- 15 "BMP-5" refers to proteins encoded by a human BMP-5 gene and defining a conserved C-terminal seven domain (SEQ ID NO: 27). The amino acid sequence for the full length protein appears in Celeste, et al. (1991) PNAS 87:9843-9847. The pro domain likely extends from the signal peptide cleavage site to residue 316; the mature protein likely is defined by residues 317-454.
- 20 "BMP-6" refers to proteins encoded by a human BMP-6 gene and defining a conserved C-terminal seven domain (SEQ ID NO: 28). The amino acid sequence for the full length protein appears in Celeste, et al. (1990) PNAS 87:9843-5847. The pro domain likely includes extends from the signal peptide cleavage site to residue 374; the mature sequence likely includes residues 375-513.
- 25 As shown in Figure 7, the OP-2 and OP-3 proteins have an additional cysteine residue in the conserved C-terminal region (e.g., see residue 41 of SEQ ID NOs: 6 and 7). The GDF-1 protein has a four amino acid insert within the conserved C-terminal cysteine domain (residues 44-47 of SEQ ID NO: 13). Further, the BMP-2 and BMP-4 proteins are missing one amino acid residue within the cysteine domain. Thus, the alignment of these amino acid

- 25 -

sequences in Figure 7 illustrates the principles of alignment used herein with respect to the preferred reference sequence of human OP-1, residues 38-139 of SEQ ID NO: 4.

In addition to the OP/BMP renal therapeutic agents described in the previous section, the present invention may be practiced using "morphogens," as defined herein. Morphogens useful in the present invention include those in which the amino acid sequences of morphogen polypeptides comprise a sequence sharing at least 70% amino acid sequence homology or "similarity", and preferably 80% homology or similarity with a reference sequence selected from the foregoing naturally OP/BMP family members. Preferably, the reference protein is human OP-1, and the reference sequence thereof is the C-terminal seven cysteine domain present in active forms of human OP-1, residues 38-139 of SEQ ID NO: 4. Morphogens useful herein accordingly include allelic, phylogenetic counterpart and other variants of the preferred reference sequence, whether naturally-occurring or biosynthetically produced (e.g., including "muteins" or "mutant proteins"), as well as novel members of the OP/BMP family of proteins set forth and identified above, e.g., in connection with Table 1. Certain particularly preferred morphogen polypeptides share at least 60% amino acid identity with the preferred reference sequence of human OP-1, still more preferably at least 65% amino acid identity therewith.

In other preferred embodiments, the morphogen polypeptides useful in the present invention are defined by a generic amino acid sequence. For example, Generic Sequence 7 (SEQ ID NO: 1) and Generic Sequence 8 (SEQ ID NO: 2) disclosed below, accommodate the homologies shared among preferred OP/BMP protein family members identified to date, including at least OP-1, OP-2, OP-3, BMP-2, BMP-3, BMP-4, 60A, DPP, Vg1, BMP-5, BMP-6, Vgr-1, and GDF-1 (SEQ ID NOS: 4-15, 24, and 26-29). The generic sequences include both the amino acid identity shared by these sequences in the C-terminal domain, defined by the six and seven cysteine domains (Generic Sequences 7 and 8, respectively), as well as alternative residues for the variable positions within the sequence. The generic sequences provide an appropriate cysteine domain where inter- or intramolecular disulfide bonds can form, and contain certain critical amino acids likely to influence the tertiary structure of the folded proteins. In addition, the generic sequences allow for an additional cysteine at position 41 (Generic Sequence 7) or position 46 (Generic Sequence 8), thereby encompassing the active sequences of OP-2 and OP-3.

30

Generic Sequence 7

| | | | | | | |
|-----|-----|-----|-----|-----|-----|-----|
| Leu | Xaa | Xaa | Xaa | Phe | Xaa | Xaa |
| 1 | | | | | 5 | |

- 26 -

| | | | | | | | | | |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Xaa | Gly | Trp | Xaa | Xaa | Xaa | Xaa | Xaa | Xaa | Pro |
| | | 10 | | | | | 15 | | |
| Xaa | Xaa | Xaa | Xaa | Ala | Xaa | Tyr | Cys | Xaa | Gly |
| | | 20 | | | | | 25 | | |
| Xaa | Cys | Xaa | Xaa | Pro | Xaa | Xaa | Xaa | Xaa | Xaa |
| | | 30 | | | | | 35 | | |
| Xaa | Xaa | Xaa | Asn | His | Ala | Xaa | Xaa | Xaa | Xaa |
| | | 40 | | | | | 45 | | |
| Xaa |
| | | 50 | | | | | 55 | | |
| Xaa | Xaa | Xaa | Cys | Cys | Xaa | Pro | Xaa | Xaa | Xaa |
| | | 60 | | | | | 65 | | |
| Xaa | Xaa | Xaa | Xaa | Xaa | Leu | Xaa | Xaa | Xaa | Xaa |
| | | 70 | | | | | 75 | | |
| Xaa | Xaa | Xaa | Val | Xaa | Leu | Xaa | Xaa | Xaa | Xaa |
| | | 80 | | | | | 85 | | |
| Xaa | Met | Xaa | Val | Xaa | Xaa | Cys | Xaa | Cys | Xaa |
| | | 90 | | | | | 95 | | |

wherein each Xaa independently is selected from a group of one or more specified amino acids defined as follows: "Res." means "residue" and Xaa at res.2 = (Tyr or Lys); Xaa at res.3 = Val or Ile); Xaa at res.4 = (Ser, Asp or Glu); Xaa at res.6 = (Arg, Gln, Ser, Lys or Ala); Xaa at res.7 = 5 (Asp or Glu); Xaa at res.8 = (Leu, Val or Ile); Xaa at res.11 = (Gln, Leu, Asp, His, Asn or Ser); Xaa at res.12 = (Asp, Arg, Asn or Glu); Xaa at res. 13 = (Trp or Ser); Xaa at res.14 = (Ile or Val); Xaa at res.15 = (Ile or Val); Xaa at res.16 (Ala or Ser); Xaa at res.18 = (Glu, Gln, Leu, Lys, Pro or Arg); Xaa at res.19 = (Gly or Ser); Xaa at res.20 = (Tyr or Phe); Xaa at res.21 = (Ala, Ser, Asp, Met, His, Gln, Leu or Gly); Xaa at res.23 = (Tyr, Asn or Phe); Xaa at res.26 = (Glu, His, 10 Tyr, Asp, Gln, Ala or Ser); Xaa at res.28 = (Glu, Lys, Asp, Gln or Ala); Xaa at res.30 = (Ala, Ser, Pro, Gln, Ile or Asn); Xaa at res.31 = (Phe, Leu or Tyr); Xaa at res.33 = (Leu, Val or Met); Xaa at res.34 = (Asn, Asp, Ala, Thr or Pro); Xaa at res.35 = (Ser, Asp, Glu, Leu, Ala or Lys); Xaa at res.36 = (Tyr, Cys, His, Ser or Ile); Xaa at res.37 = (Met, Phe, Gly or Leu); Xaa at res.38 = (Asn, Ser or Lys); Xaa at res.39 = (Ala, Ser, Gly or Pro); Xaa at res.40 = (Thr, Leu or Ser); Xaa at 15 res.44 = (Ile, Val or Thr); Xaa at res.45 = (Val, Leu, Met or Ile); Xaa at res.46 = (Gln or Arg); Xaa at res.47 = (Thr, Ala or Ser); Xaa at res.48 = (Leu or Ile); Xaa at res.49 = (Val or Met); Xaa at res.50 = (His, Asn or Arg); Xaa at res.51 = (Phe, Leu, Asn, Ser, Ala or Val); Xaa at res.52 = (Ile, Met, Asn, Ala, Val, Gly or Leu); Xaa at res.53 = (Asn, Lys, Ala, Glu, Gly or Phe); Xaa at res.54 = (Pro, Ser or Val); Xaa at res.55 = (Glu, Asp, Asn, Gly, Val, Pro or Lys); Xaa at res.56 =

- 27 -

(Thr, Ala, Val, Lys, Asp, Tyr, Ser, Gly, Ile or His); Xaa at res.57 = (Val, Ala or Ile); Xaa at res.58 = (Pro or Asp); Xaa at res.59 = (Lys, Leu or Glu); Xaa at res.60 = (Pro, Val or Ala); Xaa at res.63 = (Ala or Val); Xaa at res.65 = (Thr, Ala or Glu); Xaa at res.66 = (Gln, Lys, Arg or Glu); Xaa at res.67 = (Leu, Met or Val); Xaa at res.68 = (Asn, Ser, Asp or Gly); Xaa at res.69 = 5 (Ala, Pro or Ser); Xaa at res.70 = (Ile, Thr, Val or Leu); Xaa at res.71 = (Ser, Ala or Pro); Xaa at res.72 = (Val, Leu, Met or Ile); Xaa at res.74 = (Tyr or Phe); Xaa at res.75 = (Phe, Tyr, Leu or His); Xaa at res.76 = (Asp, Asn or Leu); Xaa at res.77 = (Asp, Glu, Asn, Arg or Ser); Xaa at res.78 = (Ser, Gln, Asn, Tyr or Asp); Xaa at res.79 = (Ser, Asn, Asp, Glu or Lys); Xaa at res.80 = (Asn, Thr or Lys); Xaa at res.82 = (Ile, Val or Asn); Xaa at res.84 = (Lys or Arg); Xaa at res.85 10 = (Lys, Asn, Gln, His, Arg or Val); Xaa at res.86 = (Tyr, Glu or His); Xaa at res.87 = (Arg, Gln, Glu or Pro); Xaa at res.88 = (Asn, Glu, Trp or Asp); Xaa at res.90 = (Val, Thr, Ala or Ile); Xaa at res.92 = (Arg, Lys, Val, Asp, Gln or Glu); Xaa at res.93 = (Ala, Gly, Glu or Ser); Xaa at res.95 = (Gly or Ala) and Xaa at res.97 = (His or Arg).

Generic Sequence 8 (SEQ ID NO: 2) includes all of Generic Sequence 7 and in addition 15 includes the following sequence (SEQ ID NO: 14) at its N-terminus:

| | | | | |
|-----|-----|-----|-----|-----|
| Cys | Xaa | Xaa | Xaa | Xaa |
| | 1 | | 5 | |

Accordingly, beginning with residue 7, each "Xaa" in Generic Sequence 8 is a specified amino acid defined as for Generic Sequence 7, with the distinction that each residue number described for Generic Sequence 7 is shifted by five in Generic Sequence 8. Thus, "Xaa at res.2 =(Tyr or Lys)" in Generic Sequence 7 refers to Xaa at res. 7 in Generic Sequence 8. In Generic Sequence 20 8, Xaa at res.2 = (Lys, Arg, Ala or Gln); Xaa at res.3 = (Lys, Arg or Met); Xaa at res.4 = (His, Arg or Gln); and Xaa at res.5 = (Glu, Ser, His, Gly, Arg, Pro, Thr, or Tyr).

As noted above, certain currently preferred morphogen polypeptide sequences useful in this invention have greater than 60% identity, preferably greater than 65% identity, with the amino acid sequence defining the preferred reference sequence of hOP-1. These particularly 25 preferred sequences include allelic and phylogenetic counterpart variants of the OP-1 and OP-2 proteins, including the Drosophila 60A protein. Accordingly, in certain particularly preferred embodiments, useful morphogens include active proteins comprising pairs of polypeptide chains within the generic amino acid sequence herein referred to as "OPX" (SEQ ID NO: 3), which defines the seven cysteine domain and accommodates the homologies between several identified

variants of OP-1 and OP-2. As described therein, each Xaa at a given position independently is selected from the residues occurring at the corresponding position in the C-terminal sequence of mouse or human OP-1 or OP-2 (see SEQ ID NOs: 4-7 and/or SEQ ID NOs: 15-22).

In still other preferred embodiments, useful morphogen polypeptides have amino acid sequences comprising a sequence encoded by a nucleic acid that hybridizes, under stringent hybridization conditions, to DNA or RNA encoding reference morphogen sequences, e.g., C-terminal sequences defining the conserved C-terminal seven domains of OP-1 or OP-2, e.g., nucleotides 1036-1341 and nucleotides 1390-1695 of SEQ ID NO: 15 and 19, respectively. As used herein, stringent hybridization conditions are defined as hybridization according to known techniques in 40% formamide, 5 X SSPE, 5 X Denhardt's Solution, and 0.1% SDS at 37°C overnight, and washing in 0.1 X SSPE, 0.1% SDS at 50°C.

As noted above, morphogens useful in the present invention generally are dimeric proteins comprising a folded pair of the above polypeptides. Morphogens are inactive when reduced, but are active as oxidized homodimers and when oxidized in combination with other morphogens of this invention to produce heterodimers. Thus, members of a folded pair of morphogen polypeptides in a morphogenically active protein can be selected independently from any of the specific morphogen polypeptides mentioned above. As noted above, a protein is morphogenic herein generally if it induces the developmental cascade of cellular and molecular events that culminate in the formation of new, organ-specific tissue. The morphogens generally are competent to induce all of the following biological functions in a morphogenically permissive environment: stimulating proliferation of progenitor cells; stimulating the differentiation of progenitor cells; stimulating the proliferation of differentiated cells; and supporting the growth and maintenance of differentiated cells.

The morphogens useful in the methods, compositions and devices of this invention include proteins comprising any of the polypeptide chains described above, whether isolated from naturally-occurring sources, or produced by recombinant DNA or other synthetic techniques, and includes allelic and phylogenetic counterpart variants of these proteins, as well as biosynthetic variants (muteins) thereof, and various truncated and fusion constructs. Deletion or addition mutants also are envisioned to be active, including those which may alter the conserved C-terminal six or seven cysteine domain, provided that the alteration does not functionally disrupt the relationship of these cysteines in the folded structure. Accordingly, such active forms are considered the equivalent of the specifically described constructs disclosed herein. The proteins

- 29 -

may include forms having varying glycosylation patterns, varying N-termini, a family of related proteins having regions of amino acid sequence homology, and active truncated or mutated forms of native or biosynthetic proteins, produced by expression of recombinant DNA in host cells.

Figure 7 herein sets forth an alignment of the amino acid sequences of the active regions of naturally occurring proteins that have been identified or appreciated herein as OP/BMP renal therapeutic agents, including human OP-1 (hOP-1, SEQ ID NOs: 4 and 15-16), mouse OP-1 (mOP-1, SEQ ID NOs: 5 and 17-18), human and mouse OP-2 (SEQ ID NOs: 6, 7, and 19-22), mouse OP-3 (SEQ ID NOs: 25-26), BMP-2 (SEQ ID NO: 8), BMP-4 (SEQ ID NO: 9), BMP-3 (SEQ ID NO: 27), DPP (from *Drosophila*, SEQ ID NO: 10), Vgl, (from *Xenopus*, SEQ ID NO: 11), Vgr-1 (from mouse, SEQ ID NO: 12), GDF-1 (from mouse and/or human, SEQ ID NOs: 13, 30 and 31), 60A protein (from *Drosophila*, SEQ ID NOs: 23 and 24), BMP-5 (SEQ ID NO: 28) and BMP-6 (SEQ ID NO: 29). The sequences are aligned essentially following the method of Needleman, et al. (1970) *J. Mol. Biol.*, 48:443-453, calculated using the Align Program (DNAstar, Inc.). In Figure 7, three dots indicates that the amino acid in that position is the same as the corresponding amino acid in hOP-1. Three dashes indicates that no amino acid is present in that position, and are included for purposes of illustrating homologies. For example, amino acid residue 60 of BMP-2 (CBMP-2A) and BMP-4 (CBMP-2B) is "missing." Of course, both of these amino acid sequences in this region comprise Asn-Ser (residues 58, 59), with BMP-2 then comprising Lys and Ile, whereas BMP-4 comprises Ser and Ile. Figure 7 also illustrates the handling of insertions in the morphogen amino acid sequence: between residues 56 and 57 of BMP-3 is an inserted Val residue; between residues 43 and 44 of GDF-1 is inserted the amino acid sequence, Gly-Gly-Pro-Pro. Such deviations from the reference morphogen sequence are ignored for purposes of calculating the defined relationship between, e.g., GDF-1 and hOP-1. As is apparent from the amino acid sequence comparisons set forth in Figure 7, significant amino acid changes can be made from the reference sequence while retaining activity. For example, while the GDF-1 protein sequence depicted in Figure 7 shares only about 50% amino acid identity with the hOP-1 sequence described therein, the GDF-1 sequence shares greater than 70% amino acid sequence homology (or "similarity") with the hOP-1 sequence, where "homology" or "similarity" includes allowed conservative amino acid substitutions within the aligned sequence, e.g., as defined by Dayhoff, et al. (1979) 5 Atlas of Protein Sequence and Structure Suppl. 3, pp. 345-362, (M.O. Dayhoff, ed., Natl. BioMed. Res. Found., Washington D.C.).

- 30 -

Accordingly, in still another preferred aspect, the invention includes morphogens comprising species of polypeptide chains having the generic amino acid sequence referred to herein as "OPX", which defines the seven cysteine domain and accommodates the identities and homologies between the various identified OP-1 and OP-2 proteins. OPX is presented in SEQ ID

- 5 NO: 3. As described therein, each Xaa at a given position independently is selected from the residues occurring at the corresponding position in the C-terminal sequence of mouse or human OP-1 or OP-2 (see Figure 7 and SEQ ID NOs: 4-7 and/or SEQ ID NOs: 15-22).

In another set of embodiments, an effective amount of an agent competent to stimulate or induce increased endogenous expression of an OP/BMP renal therapeutic agent or morphogen in 10 a mammal may be administered. For example, an agent competent to stimulate or induce OP-1 production and/or secretion from renal tissue may be provided to a mammal, e.g., by systemic administration to the mammal or by direct administration of the morphogen-stimulating agent to renal tissue. Alternatively, the morphogen-stimulating agent or "morphogen inducer" may induce morphogen expression and/or secretion at a distant site (e.g., at a tissue locus other than renal 15 tissue), with the expressed morphogen circulating to renal tissue. A method for identifying and testing agents competent to modulate the levels of endogenous morphogens in a given tissue is described in detail in published applications WO93/05172 and WO93/05751, the teachings of which are incorporated herein by reference. Briefly, candidate compounds can be identified and tested by incubation in vitro with a test tissue or cells thereof, or a cultured cell line derived 20 therefrom, for a time sufficient to allow the compound to affect the production, i.e., the expression and/or secretion, of a morphogen produced by the cells of that tissue. Here, suitable tissue, or cultured cells of a suitable tissue, preferably can be selected from renal epithelium, fibroblasts, and osteoblasts.

In another series of embodiments, an agent which acts as an agonist of an OP/BMP renal 25 therapeutic agent or morphogen receptor may be administered instead of the OP/BMP renal therapeutic agent or morphogen itself. Such an agent may also be referred to as a morphogen "mimic," "mimetic," or "analog." Thus, for example, a small peptide or other molecule which can mimic the activity of an OP/BMP renal therapeutic agent or morphogen in binding to and activating the OP/BMP renal therapeutic agent or morphogen's receptor may be employed as an 30 equivalent of the OP/BMP renal therapeutic agent or morphogen. Preferably the agonist is a full agonist, but partial receptor agonists may also be advantageously employed. Methods of identifying such agonists are known in the art and include assays for compounds which induce

- 31 -

morphogen-mediated responses (e.g., induction of differentiation of metanephric mesenchyme, induction of endochondral bone formation). For example, methods of identifying morphogen inducers or agonists of morphogen receptors may be found in U.S. Ser. No. 08/478,097 filed June 7, 1995 and U.S. Ser. No. 08/507,598 filed July 26, 1995, the disclosures of which are
5 incorporated herein by reference.

Finally, in other embodiments cells may be implanted into the kidney of a subject in, or at risk of, chronic renal failure, or at risk of needing renal replacement therapy, in order to serve as a source of an OP/BMP renal therapeutic agent or morphogen and/or to provide a source of additional functional renal tissue. Such cells may be host or donor cells which normally express
10 OP/BMP renal therapeutic agents or morphogens, which have been transformed so as to express OP/BMP renal therapeutic agents or morphogens, or which have been treated with OP/BMP renal therapeutic agents or morphogens.

D. Subjects for Treatment

As a general matter, the methods of the present invention may be utilized for any
15 mammalian subject in, or at risk of, chronic renal failure, or at risk of the need for renal replacement therapy (i.e., chronic dialysis or renal transplant). Mammalian subjects which may be treated according to the methods of the invention include, but are not limited to, human subjects or patients. In addition, however, the invention may be employed in the treatment of domesticated mammals which are maintained as human companions (e.g., dogs, cats, horses),
20 which have significant commercial value (e.g., dairy cows, beef cattle, sporting animals), which have significant scientific value (e.g., captive or free specimens of endangered species), or which otherwise have value. In addition, as a general matter, the subjects for treatment with the methods of the present invention need not present indications for treatment with an OP/BMP renal therapeutic agent or morphogen other than those indications associated with risk of chronic
25 renal failure. That is, the subjects for treatment are expected to be otherwise free of indications for treatment according to the present invention. In some number of cases, however, the subjects may present with other symptoms (e.g., osteodystrophy) for which treatment with an OP/BMP renal therapeutic agent or morphogen would be indicated. In such cases, the treatment should be adjusted accordingly so to avoid excessive dosing.

30 One of ordinary skill in the medical or veterinary arts is trained to recognize subjects which may be at a substantial risk of chronic renal failure, or at substantial risk of the need for renal replacement therapy. In particular, clinical and non-clinical trials, as well as accumulated

experience, relating to the presently disclosed and other methods of treatment, are expected to inform the skilled practitioner in deciding whether a given subject is in, or at risk of, chronic renal failure, or at risk of needing renal replacement therapy, and whether any particular treatment is best suited to the subject's needs, including treatment according to the present invention.

5 As a general matter, a mammalian subject may be regarded as being in, or at risk of, chronic renal failure, or at risk of needing renal replacement therapy, if that subject has already been diagnosed as afflicted with, or would be regarded as being afflicted with, a condition which typically leads to progressive loss of renal function associated with progressive loss of functioning nephron units. Such conditions include, but are not limited to, chronic renal failure, end-stage
10 renal disease, chronic diabetic nephropathy, hypertensive nephrosclerosis, chronic glomerulonephritis, hereditary nephritis, renal dysplasia and the like. These, and other diseases and conditions known in the art, typically lead to a progressive loss of functioning nephrons and to the onset of chronic renal failure.

15 Frequently, one of skill in the medical or veterinary arts may base a prognosis, diagnosis or treatment decision upon an examination of a renal biopsy sample. Such biopsies provide a wealth of information useful in diagnosing disorders of the kidney but, due to the invasiveness of the procedure, and the additional trauma to a presumably unhealthy kidney, may not be appropriate for all subjects. Nonetheless, subjects in, or at risk of, chronic renal failure, or at risk of needing renal replacement therapy, may be recognized by histological indications from renal
20 biopsies including, but not limited to, glomerular hypertrophy, tubular hypertrophy, glomerulosclerosis, tubulointerstitial sclerosis, and the like.

25 Less invasive techniques for assessing kidney morphology include MRI, CAT and ultrasound scans. Scanning techniques are also available which employ contrasting or imaging agents (e.g., radioactive dyes) but, it should be noted, some of these are particularly toxic to renal tissues and structures and, therefore, their use may be ill-advised in subjects in, or at risk of, chronic renal failure. Such non-invasive scanning techniques may be employed to detect conditions such as renal fibrosis or sclerosis, focal renal necrosis, renal cysts, and renal gross hypertrophy which will place a subject in, or at risk of, chronic renal failure, or at risk of needing renal replacement therapy.

30 Quite frequently, prognosis, diagnosis and/or treatment decisions are based upon clinical indications of renal function. One such indication is the presence in urinary sediment of an unusual number of "broad" or "renal failure" casts, which is indicative of tubular hypertrophy and

suggests the compensatory renal hypertrophy which typifies chronic renal failure. A better indication of renal function is the glomerular flow rate (GFR), which can be measured directly by quantifying the rate of clearance of particular markers, or which may be inferred from indirect measurements.

5 It should be noted that the present invention is not directed to the measurement of GFR or to the diagnosis of chronic renal failure. The methods of treatment of the present invention need not, therefore, be restricted to subjects presenting with any particular measures of GFR, or any other particular marker of renal function. Indeed, it is not necessary that the GFR of a subject, or any other particular marker of renal function, be determined before practicing the treatments of
10 the present invention. Nonetheless, the measurement of GFR is considered to be a preferred means of assessing renal function.

As is well known in the art, GFR reflects the rate of clearance of a reference or marker compound from the plasma to the urine. The marker compound to be considered is typically one which is freely filtered by the glomeruli, but which is not actively secreted or reabsorbed by the
15 renal tubules, and which is not significantly bound by circulating proteins. The rate of clearance is typically defined by the formula, presented above, which relates the volume of urine produced in a twenty-four period, and the relative concentrations of the marker in the urine and plasma. To be more accurate, the GFR should also be corrected for body surface area. The "gold standard" reference compound is inulin because of its filtration properties and lack of serum-binding. The
20 concentration of this compound is, however, difficult to quantify in blood or urine. The clearance rates of other compounds, including p-aminohippurate (PAH) and creatinine, are therefore often used instead of inulin. In addition, various formulas are often employed which seek to simplify the estimation of actual GFR by omitting considerations of actual urine concentrations of the marker, actual daily volumes of urine produced, or actual body surface area. These values may be
25 replaced by estimates based on other factors, by baseline values established for the same subject, or by standard values for similar subjects. These estimates should be used with caution, however, as they may entail inappropriate assumptions based upon the renal function of normal or healthy subjects.

Various methods and formulas have been developed in the art which describe an expected
30 value of GFR for a healthy subject with certain characteristics. In particular, formulas are available which provide an expected value of the GFR based upon plasma creatinine levels, age, weight and sex. One such formula for an expected GFR is presented above. Other formulas may,

of course, be employed and tables of standard values may be produced for subjects of a given age, weight, sex, and/or plasma creatinine concentration. Newer methods of measuring or estimating GFR (e.g., using NMR or MRI technologies) are also now available in the art and may be used in accordance with the present invention (see, e.g., U.S. Pat. Nos. 5,100,646 and 5,335,660).

5 As a general matter, irrespective of the manner in which GFR is measured or estimated, a subject may be considered to be in, or at risk of, chronic renal failure, or at risk of needing renal replacement therapy, when the subject has a GFR which is chronically less than about 50% of the expected GFR for that subject. The risk is considered greater as the GFR falls lower. Thus, a subject is increasingly considered at risk if the subject has a GFR which is chronically less than
10 about 40%, 30% or 20% of the expected GFR.

As a general matter, irrespective of the manner in which GFR is measured or estimated, a human male subject weighing at least about 50 kg may be considered to be in, or at risk of, chronic renal failure, or at risk of needing renal replacement therapy, when the subject has a GFR which is chronically less than about 50 ml/min. The risk is considered greater as the GFR falls
15 lower. Thus, a subject is increasingly considered at risk if the subject has a GFR which is chronically less than about 40, 30 or 20 ml/min.

As a general matter, irrespective of the manner in which GFR is measured or estimated, a human female subject weighing at least about 40 kg may be considered to be in, or at risk of, chronic renal failure, or at risk of needing renal replacement therapy, when the subject has a GFR which is chronically less than about 40 ml/min. The risk is considered greater as the GFR falls
20 lower. Thus, a subject is increasingly considered at risk if the subject has a GFR which is chronically less than about 30, 20 or 10 ml/min.

By employing a variety of methods, including the histological examinations, non-invasive scanning procedures, evaluations of clinical indicators, and other techniques described above and
25 known in the art, those in the medical and veterinary arts may provide estimates of either the number of functioning nephron units which a subject possesses, or the percentage of functioning nephron units which a subject possesses relative to a healthy but otherwise similar subject (e.g., a conspecific subject of approximately the same age, weight, and sex). Thus, for example, a biopsy may reveal a decrease in the density of functional nephrons, or imaging with filtered agents may
30 indicate losses of functional renal tissue and/or filtering capacity. Such measures or estimates provide another means of expressing when a subject is in, or at risk of, chronic renal failure, or at risk of needing renal replacement therapy. Thus, as a general matter, a subject may be regarded

to be in, or at risk of, chronic renal failure, or at risk of needing renal replacement therapy, if that subject possesses a number of functional nephron units which is less than about 50% of the number of functional nephron units of a healthy, but otherwise similar, subject. As above, the risk is considered greater as the number of functional nephrons decreases further. Thus, a subject is
5 increasingly considered at risk if the subject has a number of functional nephrons which is less than about 40, 30 or 20% of the number for a similar but healthy subject.

Finally, it should be noted that subjects possessing a single kidney, irrespective of the manner of loss of the other kidney (e.g., physical trauma, surgical removal, birth defect), may be considered to be prima facie at risk of chronic renal failure, or the need for renal replacement
10 therapy. This is particularly true for those subjects in which one kidney has been lost due to a disease or condition which may afflict the remaining kidney. Similarly, subjects which are already recipients of a renal transplant, or which are already receiving chronic dialysis (e.g., chronic hemodialysis or continuous ambulatory peritoneal dialysis) may be considered prima facie to be at risk of chronic renal failure, or the need for further renal replacement therapy.
15

E. Formulations and Methods of Treatment

The OP/BMP renal therapeutic agents, morphogens, morphogen inducers, or agonists of morphogen receptors of the present invention may be administered by any route which is compatible with the particular morphogen, inducer, or agonist employed. Thus, as appropriate, administration may be oral or parenteral, including intravenous, intraperitoneal, and renal
20 intracapsular routes of administration. In addition, administration may be by periodic injections of a bolus of the agent, or may be made more continuous by intravenous or intraperitoneal administration from a reservoir which is external (e.g., an i.v. bag) or internal (e.g., a bioerodable implant).

The therapeutic agents of the invention may be provided to an individual by any suitable
25 means, preferably directly (e.g., locally, as by injection or topical administration to a tissue locus) or systemically (e.g., parenterally or orally). Where the agent is to be provided parenterally, such as by intravenous, subcutaneous, intramuscular, intraorbital, ophthalmic, intraventricular, intracranial, intracapsular, intraspinal, intracisternal, intraperitoneal, buccal, rectal, vaginal, intranasal or by aerosol administration, the agent preferably comprises part of an aqueous
30 solution. The solution is physiologically acceptable so that in addition to delivery of the desired agent to the patient, the solution does not otherwise adversely affect the patient's electrolyte and/or volume balance. The aqueous medium for the agent thus may comprise normal

physiologic saline (e.g., 9.85% NaCl, 0.15M, pH 7-7.4). Such an aqueous solution containing the agent can be made, for example, by dissolving the agent in 50% ethanol containing acetonitrile in 0.1% trifluoroacetic acid (TFA) or 0.1% HCl, or equivalent solvents. One volume of the resultant solution then is added, for example, to ten volumes of phosphate buffered saline (PBS),
5 which further may include 0.1-0.2% human serum albumin (HSA). The resultant solution preferably is vortexed extensively.

If desired, an agent may be made more soluble by association with a suitable molecule. For example, association of the mature OP/BMP or morphogen dimer with the pro domain results in the pro form of the protein which typically is more soluble or dispersible in physiological
10 solutions than the corresponding mature form. In fact, endogenous OP/BMP proteins are thought to be transported (e.g., secreted and circulated) in the mammalian body in this form. This soluble form of the protein can be obtained from culture medium of mammalian cells, e.g., cells transfected with nucleic acid encoding and competent to express the OP/BMP protein or morphogen. Alternatively, a soluble species can be formulated by complexing the mature dimer
15 (or an active fragment thereof) with a pro domain or a solubility-enhancing fragment thereof (described more fully below). Another molecule capable of enhancing solubility and particularly useful for oral administrations, is casein. For example, addition of 0.2% casein increases solubility of the mature active form of OP-1 by 80%. Other components found in milk and/or various serum proteins also may be useful.

Finally, as noted above, in another series of embodiments renal cells may be implanted into the kidney of a subject in, or at risk of, chronic renal failure, or at risk of needing renal replacement therapy, in order to serve as a source of an OP/BMP renal therapeutic agent or morphogen and/or to provide a source of additional functional renal tissue. These cells may be any compatible mammalian cells, including renal mesenchymal progenitor cells, or renal
25 mesenchymal progenitor cells which have been induced to undergo metanephric differentiation. The cells may be derived from a donor (e.g., a tissue-type matched donor, sibling, identical twin), may be derived from a tissue culture (e.g., undifferentiated renal mesenchyme culture, fetal renal tissue culture), or may be explanted from the subject and then be re-implanted after proliferation and/or differentiation. Preferably, the cells are induced to undergo metanephric differentiation by
30 treatment with an OP/BMP renal therapeutic agent or morphogen (e.g., OP-1) either before or after implantation. Thus, for example, renal mesenchymal progenitor cells may be explanted from a subject, allowed or caused to proliferate in vitro, be induced to undergo metanephric

differentiation by morphogen treatment, and be re-implanted where they may provide a source of morphogen and/or differentiate further into functional renal tissue.

Practice of the invention, including additional preferred aspects and embodiments thereof, will be still more fully understood from the following examples, which are presented herein for 5 illustration only and should not be construed as limiting the invention in any way.

Examples

Rat Remnant Kidney Model

A rat partial (5/6) nephrectomy or rat remnant kidney model (RRKM) model was employed essentially as described (Vukicevic, et al. (1987) *J. Bone Mineral Res.* 2:533). Male 10 rats (2-3 months old, weighing about 150-200 g) were subjected to unilateral nephrectomy (either left or right kidney). After approximately one week, 2/3 of the remaining kidney was surgically removed. Immediately following surgery, plasma creatinine and BUN levels rise dramatically due to the loss of renal mass and function. Over the next several weeks of this "acute" failure phase, plasma creatinine and BUN levels of surviving animals decline somewhat toward normal values 15 but remain elevated. Renal function then appears to remain relatively constant or stable for a period of variable duration. After this point, the animals enter a period of chronic renal failure in which there is an essentially linear decline in renal function ending in death.

As surgical controls, additional rats were subjected to a "sham" operation in which the kidneys were decapsulated but no renal tissue was removed.

20 Intervention Model for Chronic Renal Failure

In this model, both nephrectomized and sham-operated rats were maintained for approximately 5-6 months after surgery. At this point, surviving nephrectomized animals were past the stable phase and had entered chronic renal failure.

Rats were divided into 8 groups with 12 rats in each group. Two groups of 25 nephrectomized rats were used as controls (Nx controls), with one of those groups receiving no treatment at all, while the other received injections of only the vehicle buffer. In addition, two groups of sham-operated rats were used as controls (sham controls), with one group receiving only the vehicle buffer, while the other received soluble OP-1 (sOP-1) at 10 µg/kg body weight. Four experimental groups of nephrectomized rats were employed, receiving sOP-1 at 1, 3, 10 or 30 50 µg/kg body weight by intraperitoneal injection (OP-1 Nx animals). OP-1 treated and vehicle-only rats received three injections per week for 4-8 weeks. Total injection volume was 300 µl.

- 38 -

No statistically significant differences were observed between the two Nx control groups or between the two sham control groups.

Compared to the sham group receiving only vehicle, the Nx control receiving only vehicle demonstrated significantly ($p < 0.01$) elevated serum creatinine (Figure 1) at the end of the study, 5 indicating a significant loss of renal function. Although nephrectomized rats treated with either 1 or 3 $\mu\text{g}/\text{kg}$ body weight sOP-1 did not show significantly reduced serum creatinine when compared to the Nx control, nephrectomized rats treated with sOP-1 at doses of 10 or 50 $\mu\text{g}/\text{kg}$ body weight showed significant ($p < 0.05$) reductions in creatinine values (Figure 1). Similar results were observed for serum urea levels: Although nephrectomized rats treated with either 1 10 or 3 $\mu\text{g}/\text{kg}$ body weight sOP-1 did not show significantly reduced serum urea when compared to the Nx control, nephrectomized rats treated with sOP-1 at doses of 10 or 50 $\mu\text{g}/\text{kg}$ body weight showed significant ($p < 0.01$) reductions in serum urea values (Figure 2). All nephrectomized rats showed significantly ($p < 0.01$) higher serum urea when compared to the sham-operated rats (Figure 2).

15 Histological observations indicate that, in contrast to the vehicle treated Nx control group, OP-1 treated nephrectomized rats exhibit relatively normal glomerular histology,. Figure 3, for example, shows typical renal samples from (A) normal rat kidney, (B) untreated Nx control animals, and (C) OP-1 treated nephrectomized rats under low magnification (10x). Figure 4 shows similar samples under higher magnification (40x). Histomorphometric analysis indicates 20 that OP-1 Nx rats showed reduced incidence of glomerular sclerosis and loop collapse, relatively scattered sclerosis and microaneurysms, and more viable glomeruli compared to Nx control rats (Table 2).

None of the rats died in any group during this study.

Prophylactic Model for Chronic Renal Failure

25 Rats were subjected to partial nephrectomies or sham-operated as described above. In this model, in order to test the ability of OP/BMP renal therapeutic agents to prevent, inhibit or delay the initiation of chronic renal failure, the rats were allowed to recover for approximately two weeks after surgery before initiation of OP-1 therapy. At this point, surviving animals were past the acute renal failure phase and had not yet entered chronic renal failure.

30 Rats were divided into two groups of 15-20 rats. One group received only vehicle buffer (Nx control) whereas the other received OP-1 treatment at 10 $\mu\text{g}/\text{kg}$ body weight given

intraperitoneally three times per week. Administration of OP-1 or vehicle continued for a period of approximately 8-9 weeks.

During weeks 1-5 of treatment, both groups showed elevated serum creatinine ($> 100 \mu\text{mol/L}$) relative to sham-operated controls ($35 \pm 7 \mu\text{mol/L}$). At about 5 weeks, both groups
5 began to show a rise in serum creatinine suggesting the onset of progressive or chronic renal failure. The rise in serum creatinine was, however, markedly less rapid in the OP-1 treated group and was significantly lower than in the Nx controls (Figure 5: $p < 0.02$ at weeks 6 and 8; $p < 0.01$ at weeks 7 and 9). Similar results were observed in serum BUN values as well.

More important, measurements of GFR, based on serum and urine creatinine values,
10 showed a highly significant decrease in both groups of nephrectomized rats ($< 1.8 \text{ ml/min}$) relative to sham-operated controls ($4.7 \pm 1.1 \text{ ml/min}$). The GFR in both groups continued to decline during weeks 1-3 of treatment. At approximately three weeks, however, GFR in the OP-1 treated group stabilized whereas the decline in renal function continued in the Nx controls. By week 5, the difference in GFR values between OP-1 treated and Nx control rats had become statistically
15 significant ($p < 0.02$). This difference in GFR continued to increase over time ($p < 0.01$ at week 6; $p < 0.001$ at weeks 7 and 8), as the Nx controls continued to decline but the OP-1 treated rats remained stable (Figure 6). By the end of 9 weeks, 40% of the Nx control rats were dead whereas none of the OP-1 treated rats had died.

Histological evaluation of tissue sections confirmed that OP-1 treated rats showed greater
20 preservation or maintenance of glomeruli, as well as proximal and distal tubule structures. There were also signs in the OP-1 treated rats of nephrogenic mesenchymal condensations and the appearance of developmental nephrogenic structures. Table 2 reports results of several standard quantitative (e.g., PAS-staining of extracellular matrix) and semi-quantitative (e.g., visual ranking) histomorphometric measures obtained for tissue slices from Nx control and OP-1 treated
25 Nx rats. These results indicate that OP-1 treatment of nephrectomized rates resulted in overall improvement (or reduced degeneration) of kidney tissue morphology, increased mesangial or perivascular thickening, decreased glomerular sclerosis and loop collapse, decreased presence of "scattered" sclerosis and microaneurysms, and an increase in viable glomeruli.

- 40 -

TABLE 2

| Group | Normal Histology | Mesangial Thickening | Glomerular Sclerosis & Loop Collapse | Scattered Sclerosis & Microaneurysms | Absence of Viable Glomeruli |
|-------------------|------------------|----------------------|--------------------------------------|--------------------------------------|-----------------------------|
| Control (N=15) | 2.58 ±0.22 | 27.3 ±2.4 | 26.5±3.5 | 34.7±4.2 | 8.9±0.7 |
| OP-1 (N=20) | 11.41±1.1 | 58.6±3.2 | 14.7±1.3 | 11.8±1.1 | 2.5±0.2 |
| Significance | p <0.01 | p <0.01 | p <0.02 | p <0.01 | p <0.01 |

Equivalents

The invention may be embodied in other specific forms without departing from the spirit or essential characteristics thereof. The foregoing embodiments are therefore to be considered in all respects illustrative rather than limiting on the invention described herein. Scope of the invention is thus indicated by the appended claims rather than by the foregoing description, and all changes which come within the meaning and range of equivalency of the claims are intended to be embraced therein.

- 41 -

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT:

- (A) NAME: CREATIVE BIOMOLECULES, INC.
- (B) STREET: 45 SOUTH STREET
- (C) CITY: HOPKINTON
- (D) STATE: MA
- (E) COUNTRY: USA
- (F) POSTAL CODE (ZIP): 01748
- (G) TELEPHONE: 1-508-435-9001
- (H) TELEFAX: 1-508-435-0454
- (I) TELEX:

(ii) TITLE OF INVENTION: MORPHOGEN TREATMENT FOR CHRONIC
RENAL FAILURE

(iii) NUMBER OF SEQUENCES: 31

(iv) CORRESPONDENCE ADDRESS:

- (A) ADDRESSEE: CREATIVE BIOMOLECULES, INC.
- (B) STREET: 45 SOUTH STREET
- (C) CITY: HOPKINTON
- (D) STATE: MA
- (E) COUNTRY: USA
- (F) ZIP: 01748

(v) COMPUTER READABLE FORM:

- (A) MEDIUM TYPE: Floppy disk
- (B) COMPUTER: IBM PC compatible
- (C) OPERATING SYSTEM: PC-DOS/MS-DOS
- (D) SOFTWARE: PatentIn Release #1.0, Version #1.25

(vi) CURRENT APPLICATION DATA:

- (A) APPLICATION NUMBER:
- (B) FILING DATE:
- (C) CLASSIFICATION:

(vii) PRIOR APPLICATION DATA:

- (A) APPLICATION NUMBER: US 08/643,321
- (B) FILING DATE: 06-MAY-1996

(viii) ATTORNEY/AGENT INFORMATION:

- (A) NAME: TWOMEY, MICHAEL J
- (B) REGISTRATION NUMBER: 38,349
- (C) REFERENCE/DOCKET NUMBER: CRP-118PC

(ix) TELECOMMUNICATION INFORMATION:

- (A) TELEPHONE: 617/248-7000
- (B) TELEFAX: 617/248-7100

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 97 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

- 42 -

(ix) FEATURE:

- (A) NAME/KEY: Protein
- (B) LOCATION: 1..97
- (D) OTHER INFORMATION: /label= Generic-Seq-7
/note= "wherein each Xaa is independently selected from a group of one or more specified amino acids as defined in the specification."

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

Leu Xaa Xaa Xaa Phe Xaa Xaa Xaa Gly Trp Xaa Xaa Xaa Xaa Xaa Xaa
1 5 10 15

Pro Xaa Xaa Xaa Xaa Ala Xaa Tyr Cys Xaa Gly Xaa Cys Xaa Xaa Pro
20 25 30

Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Asn His Ala Xaa Xaa Xaa Xaa Xaa
35 40 45

Xaa Cys Cys Xaa Pro
50 55 60

Xaa Xaa Xaa Xaa Xaa Xaa Xaa Leu Xaa Xaa Xaa Xaa Xaa Xaa Xaa
65 70 75 80

Val Xaa Leu Xaa Xaa Xaa Xaa Met Xaa Val Xaa Xaa Cys Xaa Cys
85 90 95

Xaa

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 102 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(ix) FEATURE:

- (A) NAME/KEY: Protein
- (B) LOCATION: 1..102
- (D) OTHER INFORMATION: /label= Generic-Seq-8
/note= "wherin each Xaa is independently selected from a group of one or more specified amino acids as defined in the specification."

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Cys Xaa Xaa Xaa Xaa Leu Xaa Xaa Xaa Phe Xaa Xaa Xaa Gly Trp Xaa
1 5 10 15

Xaa Xaa Xaa Xaa Pro Xaa Xaa Xaa Ala Xaa Tyr Cys Xaa Gly

- 43 -

| | | |
|----|----|----|
| 20 | 25 | 30 |
|----|----|----|

| | | |
|---|----|----|
| Xaa Cys Xaa Xaa Pro Xaa Xaa Xaa Xaa Xaa Xaa Xaa Asn His Ala | | |
| 35 | 40 | 45 |

| | | |
|---|----|----|
| Xaa | | |
| 50 | 55 | 60 |

| | | |
|---|----|----|
| Xaa Cys Cys Xaa Pro Xaa Xaa Xaa Xaa Xaa Xaa Xaa Leu Xaa Xaa | | |
| 65 | 70 | 75 |
| 80 | | |

| | | |
|---|----|----|
| Xaa Xaa Xaa Xaa Xaa Val Xaa Leu Xaa Xaa Xaa Xaa Met Xaa Val | | |
| 85 | 90 | 95 |

| | | |
|-------------------------|--|--|
| Xaa Xaa Cys Xaa Cys Xaa | | |
| 100 | | |

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 102 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(ix) FEATURE:

- (A) NAME/KEY: Protein
- (B) LOCATION: 1..102
- (D) OTHER INFORMATION: /label= OPX
/note= "WHEREIN EACH XAA IS INDEPENDENTLY SELECTED FROM A GROUP OF ONE OR MORE SPECIFIED AMINO ACIDS AS DEFINED IN THE SPECIFICATION"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

| | | |
|---|---|----|
| Cys Xaa Xaa His Glu Leu Tyr Val Xaa Phe Xaa Asp Leu Gly Trp Xaa | | |
| 1 | 5 | 10 |
| 15 | | |

| | | |
|---|----|----|
| Asp Trp Xaa Ile Ala Pro Xaa Gly Tyr Xaa Ala Tyr Tyr Cys Glu Gly | | |
| 20 | 25 | 30 |

| | | |
|---|----|----|
| Glu Cys Xaa Phe Pro Leu Xaa Ser Xaa Met Asn Ala Thr Asn His Ala | | |
| 35 | 40 | 45 |

| | | |
|---|----|----|
| Ile Xaa Gln Xaa Leu Val His Xaa Xaa Xaa Pro Xaa Xaa Val Pro Lys | | |
| 50 | 55 | 60 |

| | | |
|---|----|----|
| Xaa Cys Cys Ala Pro Thr Xaa Leu Xaa Ala Xaa Ser Val Leu Tyr Xaa | | |
| 65 | 70 | 75 |
| 80 | | |

| | | |
|---|----|----|
| Asp Xaa Ser Xaa Asn Val Xaa Leu Xaa Lys Xaa Arg Asn Met Val Val | | |
| 85 | 90 | 95 |

- 44 -

Xaa Ala Cys Gly Cys His
100

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 139 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Homo sapiens
(F) TISSUE TYPE: HIPPOCAMPUS

(ix) FEATURE:
(A) NAME/KEY: Protein
(B) LOCATION: 1..139
(D) OTHER INFORMATION: /label= hOP1-MATURE

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

Ser Thr Gly Ser Lys Gln Arg Ser Gln Asn Arg Ser Lys Thr Pro Lys
1 5 10 15

Asn Gln Glu Ala Leu Arg Met Ala Asn Val Ala Glu Asn Ser Ser Ser
20 25 30

Asp Gln Arg Gln Ala Cys Lys Lys His Glu Leu Tyr Val Ser Phe Arg
35 40 45

Asp Leu Gly Trp Gln Asp Trp Ile Ile Ala Pro Glu Gly Tyr Ala Ala
50 55 60

Tyr Tyr Cys Glu Gly Glu Cys Ala Phe Pro Leu Asn Ser Tyr Met Asn
65 70 75 80

Ala Thr Asn His Ala Ile Val Gln Thr Leu Val His Phe Ile Asn Pro
85 90 95

Glu Thr Val Pro Lys Pro Cys Cys Ala Pro Thr Gln Leu Asn Ala Ile
100 105 110

Ser Val Leu Tyr Phe Asp Asp Ser Ser Asn Val Ile Leu Lys Lys Tyr
115 120 125

Arg Asn Met Val Val Arg Ala Cys Gly Cys His
130 135

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:

- 45 -

- (A) LENGTH: 139 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: MURIDAE
- (F) TISSUE TYPE: EMBRYO

(ix) FEATURE:

- (A) NAME/KEY: Protein
- (B) LOCATION: 1..139
- (D) OTHER INFORMATION: /label= MOP1-MATURE

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

Ser Thr Gly Gly Lys Gln Arg Ser Gln Asn Arg Ser Lys Thr Pro Lys
1 5 10 15

Asn Gln Glu Ala Leu Arg Met Ala Ser Val Ala Glu Asn Ser Ser Ser
20 25 30

Asp Gln Arg Gln Ala Cys Lys Lys His Glu Leu Tyr Val Ser Phe Arg
35 40 45

Asp Leu Gly Trp Gln Asp Trp Ile Ile Ala Pro Glu Gly Tyr Ala Ala
50 55 60

Tyr Tyr Cys Glu Gly Glu Cys Ala Phe Pro Leu Asn Ser Tyr Met Asn
65 70 75 80

Ala Thr Asn His Ala Ile Val Gln Thr Leu Val His Phe Ile Asn Pro
85 90 95

Asp Thr Val Pro Lys Pro Cys Cys Ala Pro Thr Gln Leu Asn Ala Ile
100 105 110

Ser Val Leu Tyr Phe Asp Asp Ser Ser Asn Val Ile Leu Lys Lys Tyr
115 120 125

Arg Asn Met Val Val Arg Ala Cys Gly Cys His
130 135

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 139 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(vi) ORIGINAL SOURCE:

- 46 -

(A) ORGANISM: HOMO SAPIENS
(F) TISSUE TYPE: HIPPOCAMPUS

(ix) FEATURE:

(A) NAME/KEY: Protein
(B) LOCATION: 1..139
(D) OTHER INFORMATION: /label= HOP2-MATURE

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

Ala Val Arg Pro Leu Arg Arg Arg Gln Pro Lys Lys Ser Asn Glu Leu
1 5 10 15

Pro Gln Ala Asn Arg Leu Pro Gly Ile Phe Asp Asp Val His Gly Ser
20 25 30

His Gly Arg Gln Val Cys Arg Arg His Glu Leu Tyr Val Ser Phe Gln
35 40 45

Asp Leu Gly Trp Leu Asp Trp Val Ile Ala Pro Gln Gly Tyr Ser Ala
50 55 60

Tyr Tyr Cys Glu Gly Glu Cys Ser Phe Pro Leu Asp Ser Cys Met Asn
65 70 80

Ala Thr Asn His Ala Ile Leu Gln Ser Leu Val His Leu Met Lys Pro
85 90 95

Asn Ala Val Pro Lys Ala Cys Cys Ala Pro Thr Lys Leu Ser Ala Thr
100 105 110

Ser Val Leu Tyr Tyr Asp Ser Ser Asn Asn Val Ile Leu Arg Lys His
115 120 125

Arg Asn Met Val Val Lys Ala Cys Gly Cys His
130 135

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 139 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(vi) ORIGINAL SOURCE:

(A) ORGANISM: MURIDAE
(F) TISSUE TYPE: EMBRYO

(ix) FEATURE:

(A) NAME/KEY: Protein
(B) LOCATION: 1..139
(D) OTHER INFORMATION: /label= MOP2-MATURE

- 47 -

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

```

Ala Ala Arg Pro Leu Lys Arg Arg Gln Pro Lys Lys Thr Asn Glu Leu
1           5           10          15

Pro His Pro Asn Lys Leu Pro Gly Ile Phe Asp Asp Gly His Gly Ser
20          25          30

Arg Gly Arg Glu Val Cys Arg Arg His Glu Leu Tyr Val Ser Phe Arg
35          40          45

Asp Leu Gly Trp Leu Asp Trp Val Ile Ala Pro Gln Gly Tyr Ser Ala
50          55          60

Tyr Tyr Cys Glu Gly Glu Cys Ala Phe Pro Leu Asp Ser Cys Met Asn
65          70          75          80

Ala Thr Asn His Ala Ile Leu Gln Ser Leu Val His Leu Met Lys Pro
85          90          95

Asp Val Val Pro Lys Ala Cys Cys Ala Pro Thr Lys Leu Ser Ala Thr
100         105         110

Ser Val Leu Tyr Tyr Asp Ser Ser Asn Asn Val Ile Leu Arg Lys His
115         120         125

Arg Asn Met Val Val Lys Ala Cys Gly Cys His
130         135

```

(2) INFORMATION FOR SEQ ID NO:8:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 101 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: bovinae

(ix) FEATURE:

- (A) NAME/KEY: Protein
- (B) LOCATION: 1..101
- (D) OTHER INFORMATION: /label= CBMP-2A-FX

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

```

Cys Lys Arg His Pro Leu Tyr Val Asp Phe Ser Asp Val Gly Trp Asn
1           5           10          15

Asp Trp Ile Val Ala Pro Pro Gly Tyr His Ala Phe Tyr Cys His Gly
20          25          30

```

- 48 -

(2) INFORMATION FOR SEQ ID NO:9:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 101 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear
 - (ii) MOLECULE TYPE: protein
 - (vi) ORIGINAL SOURCE:
 - (A) ORGANISM: HOMO SAPIENS
 - (F) TISSUE TYPE: hippocampus
 - (ix) FEATURE:
 - (A) NAME/KEY: Protein
 - (B) LOCATION: 1..101
 - (D) OTHER INFORMATION: /label= CBMP-2B-FX

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

Cys Arg Arg His Ser Leu Tyr Val Asp Phe Ser Asp Val Gly Trp Asn
 1 5 10 15

 Asp Trp Ile Val Ala Pro Pro Gly Tyr Gln Ala Phe Tyr Cys His Gly
 20 25 30

 Asp Cys Pro Phe Pro Leu Ala Asp His Leu Asn Ser Thr Asn His Ala
 35 40 45

 Ile Val Gln Thr Leu Val Asn Ser Val Asn Ser Ser Ile Pro Lys Ala
 50 55 60

 Cys Cys Val Pro Thr Glu Leu Ser Ala Ile Ser Met Leu Tyr Leu Asp
 65 70 75 80

 Glu Tyr Asp Lys Val Val Leu Lys Asn Tyr Gln Glu Met Val Val Glu
 85 90 95

 Gly Cys Gly Cys Arg
 100

- 49 -

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 102 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: DROSOPHILA MELANOGASTER

(ix) FEATURE:

- (A) NAME/KEY: Protein
- (B) LOCATION: 1..101
- (D) OTHER INFORMATION: /label= DPP-FX

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

Cys Arg Arg His Ser Leu Tyr Val Asp Phe Ser Asp Val Gly Trp Asp
1 5 10 15

Asp Trp Ile Val Ala Pro Leu Gly Tyr Asp Ala Tyr Tyr Cys His Gly
20 25 30

Lys Cys Pro Phe Pro Leu Ala Asp His Phe Asn Ser Thr Asn His Ala
35 40 45

Val Val Gln Thr Leu Val Asn Asn Asn Pro Gly Lys Val Pro Lys
50 55 60

Ala Cys Cys Val Pro Thr Gln Leu Asp Ser Val Ala Met Leu Tyr Leu
65 70 75 80

Asn Asp Gln Ser Thr Val Val Leu Lys Asn Tyr Gln Glu Met Thr Val
85 90 95

Val Gly Cys Gly Cys Arg
100

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 102 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: XENOPUS

(ix) FEATURE:

- 50 -

- (A) NAME/KEY: Prot in
- (B) LOCATION: 1..102
- (D) OTHER INFORMATION: /label= VGL-FX

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

| | | | | | | | | | | | | | | | |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Cys | Lys | Lys | Arg | His | Leu | Tyr | Val | Glu | Phe | Lys | Asp | Val | Gly | Trp | Gln |
| 1 | | | | | 5 | | | 10 | | | | | | 15 | |
| Asn | Trp | Val | Ile | Ala | Pro | Gln | Gly | Tyr | Met | Ala | Asn | Tyr | Cys | Tyr | Gly |
| | 20 | | | | | 25 | | | | | 30 | | | | |
| Glu | Cys | Pro | Tyr | Pro | Leu | Thr | Glu | Ile | Leu | Asn | Gly | Ser | Asn | His | Ala |
| | 35 | | | | | 40 | | | | 45 | | | | | |
| Ile | Leu | Gln | Thr | Leu | Val | His | Ser | Ile | Glu | Pro | Glu | Asp | Ile | Pro | Leu |
| | 50 | | | | 55 | | | | 60 | | | | | | |
| Pro | Cys | Cys | Val | Pro | Thr | Lys | Met | Ser | Pro | Ile | Ser | Met | Leu | Phe | Tyr |
| 65 | | | | 70 | | | 75 | | | | | 80 | | | |
| Asp | Asn | Asn | Asp | Asn | Val | Val | Leu | Arg | His | Tyr | Glu | Asn | Met | Ala | Val |
| | 85 | | | | 90 | | | 90 | | 95 | | | | | |
| Asp | Glu | Cys | Gly | Cys | Arg | | | | | | | | | | |
| | 100 | | | | | | | | | | | | | | |

(2) INFORMATION FOR SEQ ID NO:12:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 102 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: protein
- (vi) ORIGINAL SOURCE:
 - (A) ORGANISM: MURIDAE
- (ix) FEATURE:
 - (A) NAME/KEY: Protein
 - (B) LOCATION: 1..102
 - (D) OTHER INFORMATION: /label= VGR-1-FX

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

| | | | | | | | | | | | | | | | |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Cys | Lys | Lys | His | Glu | Leu | Tyr | Val | Ser | Phe | Gln | Asp | Val | Gly | Trp | Gln |
| 1 | | | | | 5 | | | 10 | | | | | | 15 | |
| Asp | Trp | Ile | Ile | Ala | Pro | Lys | Gly | Tyr | Ala | Ala | Asn | Tyr | Cys | Asp | Gly |
| | 20 | | | | | 25 | | | | | 30 | | | | |
| Glu | Cys | Ser | Phe | Pro | Leu | Asn | Ala | His | Met | Asn | Ala | Thr | Asn | His | Ala |
| | 35 | | | | 40 | | | 40 | | 45 | | | | | |

- 51 -

Ile Val Gln Thr Leu Val His Val Met Asn Pro Glu Tyr Val Pro Lys
 50 55 60

Pro Cys Cys Ala Pro Thr Lys Val Asn Ala Ile Ser Val Leu Tyr Phe
 65 70 75 80

Asp Asp Asn Ser Asn Val Ile Leu Lys Lys Tyr Arg Asn Met Val Val
 85 90 95

Arg Ala Cys Gly Cys His
 100

(2) INFORMATION FOR SEO ID NO:13:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 106 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear
 - (ii) MOLECULE TYPE: protein
 - (iii) HYPOTHETICAL: NO
 - (iv) ANTI-SENSE: NO
 - (v) ORIGINAL SOURCE:
 - (A) ORGANISM: Homo sapiens
 - (F) TISSUE TYPE: brain
 - (ix) FEATURE:
 - (A) NAME/KEY: Protein
 - (B) LOCATION: 1..106
 - (D) OTHER INFORMATION: /note= "GDF-1 (fx)"
 - (xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

| | | | | | | | | | | | | | | | |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Cys | Arg | Ala | Arg | Arg | Leu | Tyr | Val | Ser | Phe | Arg | Glu | Val | Gly | Trp | His |
| 1 | | | | | 5 | | | | | 10 | | | | 15 | |
| Arg | Trp | Val | Ile | Ala | Pro | Arg | Gly | Phe | Leu | Ala | Asn | Tyr | Cys | Gln | Gly |
| | | | | | | | | | 20 | | | 25 | | | 30 |
| Gln | Cys | Ala | Leu | Pro | Val | Ala | Leu | Ser | Gly | Ser | Gly | Gly | Pro | Pro | Ala |
| | | | | | | | | | 35 | | | 40 | | | 45 |
| Leu | Asn | His | Ala | Val | Leu | Arg | Ala | Leu | Met | His | Ala | Ala | Ala | Pro | Gly |
| | | | | | | | | | 50 | | | 55 | | | 60 |
| Ala | Ala | Asp | Leu | Pro | Cys | Cys | Val | Pro | Ala | Arg | Leu | Ser | Pro | Ile | Ser |
| | | | | | | | | | 65 | | | 70 | | | 75 |
| Val | Leu | Phe | Phe | Asp | Asn | Ser | Asp | Asn | Val | Val | Leu | Arg | Gln | Tyr | Glu |
| | | | | | | | | | 85 | | | 90 | | | 95 |

- 52 -

Asp Met Val Val Asp Glu Cys Gly Cys Arg
100 105

(2) INFORMATION FOR SEQ ID NO:14:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 5 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

Cys Xaa Xaa Xaa Xaa
1 5

(2) INFORMATION FOR SEQ ID NO:15:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1822 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

- (vi) ORIGINAL SOURCE:
(A) ORGANISM: HOMO SAPIENS
(F) TISSUE TYPE: HIPPOCAMPUS

- (ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 49..1341
(C) IDENTIFICATION METHOD: experimental
(D) OTHER INFORMATION: /function= "OSTEOGENIC PROTEIN"
/product= "OP1"
/evidence= EXPERIMENTAL
/standard_name= "OP1"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

GGTGCGGGCC CGGAGCCCGG AGCCCCGGTA GCGCGTAGAG CCGGCGCG ATG CAC GTG 57
Met His Val
1

CGC TCA CTG CGA GCT GCG GCG CCG CAC AGC TTC GTG GCG CTC TGG GCA 105
Arg Ser Leu Arg Ala Ala Ala Pro His Ser Phe Val Ala Leu Trp Ala
5 10 15

- 53 -

| | |
|---|-----|
| CCC CTG TTC CTG CTG CGC TCC GCC CTG GCC GAC TTC AGC CTG GAC AAC Pro Leu Phe Leu Leu Arg Ser Ala Leu Ala Asp Phe Ser Leu Asp Asn 20 25 30 35 | 153 |
| GAG GTG CAC TCG AGC TTC ATC CAC CGG CGC CTC CGC AGC CAG GAG CGG Glu Val His Ser Ser Phe Ile His Arg Arg Leu Arg Ser Gln Glu Arg 40 45 50 | 201 |
| CGG GAG ATG CAG CGC GAG ATC CTC TCC ATT TTG GGC TTG CCC CAC CGC Arg Glu Met Gln Arg Glu Ile Leu Ser Ile Leu Gly Leu Pro His Arg 55 60 65 | 249 |
| CCG CGC CCG CAC CTC CAG GGC AAG CAC AAC TCG GCA CCC ATG TTC ATG Pro Arg Pro His Leu Gln Gly Lys His Asn Ser Ala Pro Met Phe Met 70 75 80 | 297 |
| CTG GAC CTG TAC AAC GCC ATG GCG GTG GAG GAG GGC GGC GGG CCC GGC Leu Asp Leu Tyr Asn Ala Met Ala Val Glu Glu Gly Gly Pro Gly 85 90 95 | 345 |
| GGC CAG GGC TTC TCC TAC CCC TAC AAG GCC GTC TTC AGT ACC CAG GGC Gly Gln Gly Phe Ser Tyr Pro Tyr Lys Ala Val Phe Ser Thr Gln Gly 100 105 110 115 | 393 |
| CCC CCT CTG GCC AGC CTG CAA GAT AGC CAT TTC CTC ACC GAC GCC GAC Pro Pro Leu Ala Ser Leu Gln Asp Ser His Phe Leu Thr Asp Ala Asp 120 125 130 | 441 |
| ATG GTC ATG AGC TTC GTC AAC CTC GTG GAA CAT GAC AAG GAA TTC TTC Met Val Met Ser Phe Val Asn Leu Val Glu His Asp Lys Glu Phe Phe 135 140 145 | 489 |
| CAC CCA CGC TAC CAC CAT CGA GAG TTC CGG TTT GAT CTT TCC AAG ATC His Pro Arg Tyr His His Arg Glu Phe Arg Phe Asp Leu Ser Lys Ile 150 155 160 | 537 |
| CCA GAA GGG GAA GCT GTC ACG GCA GCC GAA TTC CGG ATC TAC AAG GAC Pro Glu Gly Glu Ala Val Thr Ala Ala Glu Phe Arg Ile Tyr Lys Asp 165 170 175 | 585 |
| TAC ATC CGG GAA CGC TTC GAC AAT GAG ACG TTC CGG ATC AGC GTT TAT Tyr Ile Arg Glu Arg Phe Asp Asn Glu Thr Phe Arg Ile Ser Val Tyr 180 185 190 195 | 633 |
| CAG GTG CTC CAG GAG CAC TTG GGC AGG GAA TCG GAT CTC TTC CTG CTC Gln Val Leu Gln Glu His Leu Gly Arg Glu Ser Asp Leu Phe Leu Leu 200 205 210 | 681 |
| GAC AGC CGT ACC CTC TGG GCC TCG GAG GAG GGC TGG CTG GTG TTT GAC Asp Ser Arg Thr Leu Trp Ala Ser Glu Glu Gly Trp Leu Val Phe Asp 215 220 225 | 729 |
| ATC ACA GCC ACC AGC AAC CAC TGG GTG GTC AAT CCG CGG CAC AAC CTG Ile Thr Ala Thr Ser Asn His Trp Val Val Asn Pro Arg His Asn Leu 230 235 240 | 777 |

- 54 -

| | |
|---|------|
| GGC CTG CAG CTC TCG GTG GAG ACG CTG GAT GGG CAG AGC ATC AAC CCC Gly Leu Gln Leu Ser Val Glu Thr Leu Asp Gly Gln Ser Ile Asn Pro 245 250 255 | 825 |
| AAG TTG GCG GGC CTG ATT GGG CGG CAC GGG CCC CAG AAC AAG CAG CCC Lys Leu Ala Gly Leu Ile Gly Arg His Gly Pro Gln Asn Lys Gln Pro 260 265 270 275 | 873 |
| TTC ATG GTG GCT TTC TTC AAG GCC ACG GAG GTC CAC TTC CGC AGC ATC Phe Met Val Ala Phe Phe Lys Ala Thr Glu Val His Phe Arg Ser Ile 280 285 290 | 921 |
| CGG TCC ACG GGG AGC AAA CAG CGC AGC CAG AAC CGC TCC AAG ACG CCC Arg Ser Thr Gly Ser Lys Gln Arg Ser Gln Asn Arg Ser Lys Thr Pro 295 300 305 | 969 |
| AAG AAC CAG GAA GCC CTG CGG ATG GCC AAC GTG GCA GAG AAC AGC AGC Lys Asn Gln Glu Ala Leu Arg Met Ala Asn Val Ala Glu Asn Ser Ser 310 315 320 | 1017 |
| AGC GAC CAG AGG CAG GCC TGT AAG AAG CAC GAG CTG TAT GTC AGC TTC Ser Asp Gln Arg Gln Ala Cys Lys Lys His Glu Leu Tyr Val Ser Phe 325 330 335 | 1065 |
| CGA GAC CTG GGC TGG CAG GAC TGG ATC ATC GCG CCT GAA GGC TAC GCC Arg Asp Leu Gly Trp Gln Asp Trp Ile Ile Ala Pro Glu Gly Tyr Ala 340 345 350 355 | 1113 |
| GCC TAC TAC TGT GAG GGG GAG TGT GCC TTC CCT CTG AAC TCC TAC ATG Ala Tyr Tyr Cys Glu Gly Glu Cys Ala Phe Pro Leu Asn Ser Tyr Met 360 365 370 | 1161 |
| AAC GCC ACC AAC CAC GCC ATC GTG CAG ACG CTG GTC CAC TTC ATC AAC Asn Ala Thr Asn His Ala Ile Val Gln Thr Leu Val His Phe Ile Asn 375 380 385 | 1209 |
| CCG GAA ACG GTG CCC AAG CCC TGC TGT GCG CCC ACG CAG CTC AAT GCC Pro Glu Thr Val Pro Lys Pro Cys Cys Ala Pro Thr Gln Leu Asn Ala 390 395 400 | 1257 |
| ATC TCC GTC CTC TAC TTC GAT GAC AGC TCC AAC GTC ATC CTG AAG AAA Ile Ser Val Leu Tyr Phe Asp Asp Ser Ser Asn Val Ile Leu Lys Lys 405 410 415 | 1305 |
| TAC AGA AAC ATG GTG GTC CGG GCC TGT GGC TGC CAC TAGCTCCTCC Tyr Arg Asn Met Val Val Arg Ala Cys Gly Cys His 420 425 430 | 1351 |
| GAGAATTCAAG ACCCTTTGGG GCCAAGTTTT TCTGGATCCT CCATTGCTCG CCTTGGCCAG | 1411 |
| GAACCAGCAG ACCAACTGCC TTTTGTGAGA CCTTCCCCTC CCTATCCCCA ACTTTAAAGG | 1471 |
| TGTGAGAGTA TTAGGAAACA TGAGCAGCAT ATGGCTTTG ATCAGTTTT CAGTGGCAGC | 1531 |
| ATCCAATGAA CAAGATCCTA CAAGCTGTGC AGGCAGAACC TAGCAGGAAA AAAAAACAAC | 1591 |
| GCATAAAAGAA AAATGGCCGG GCCAGGTCAT TGGCTGGAA GTCTCAGCCA TGCACGGACT | 1651 |

- 55 -

| | |
|---|------|
| CGTTTCCAGA GGTAAATTATG AGCCGCCTACC AGCCAGGCCA CCCAGCCGTG GGAGGAAGGG | 1711 |
| GGCGTGGCAA GGGGTGGCA CATTGGTGTC TGTGCGAAAG GAAAATTGAC CCGGAAGTTC | 1771 |
| CTGTAATAAA TGTCAACAATA AAACGAATGA ATGAAAAAAA AAAAAAAAAA A | 1822 |

(2) INFORMATION FOR SEQ ID NO:16:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 431 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

| | | | |
|---|-----|-----|-----|
| Met His Val Arg Ser Leu Arg Ala Ala Ala Pro His Ser Phe Val Ala | | | |
| 1 | 5 | 10 | 15 |
| Leu Trp Ala Pro Leu Phe Leu Leu Arg Ser Ala Leu Ala Asp Phe Ser | | | |
| 20 | 25 | 30 | |
| Leu Asp Asn Glu Val His Ser Ser Phe Ile His Arg Arg Leu Arg Ser | | | |
| 35 | 40 | 45 | |
| Gln Glu Arg Arg Glu Met Gln Arg Glu Ile Leu Ser Ile Leu Gly Leu | | | |
| 50 | 55 | 60 | |
| Pro His Arg Pro Arg Pro His Leu Gln Gly Lys His Asn Ser Ala Pro | | | |
| 65 | 70 | 75 | 80 |
| Met Phe Met Leu Asp Leu Tyr Asn Ala Met Ala Val Glu Glu Gly Gly | | | |
| 85 | 90 | 95 | |
| Gly Pro Gly Gly Gln Gly Phe Ser Tyr Pro Tyr Lys Ala Val Phe Ser | | | |
| 100 | 105 | 110 | |
| Thr Gln Gly Pro Pro Leu Ala Ser Leu Gln Asp Ser His Phe Leu Thr | | | |
| 115 | 120 | 125 | |
| Asp Ala Asp Met Val Met Ser Phe Val Asn Leu Val Glu His Asp Lys | | | |
| 130 | 135 | 140 | |
| Glu Phe Phe His Pro Arg Tyr His His Arg Glu Phe Arg Phe Asp Leu | | | |
| 145 | 150 | 155 | 160 |
| Ser Lys Ile Pro Glu Gly Glu Ala Val Thr Ala Ala Glu Phe Arg Ile | | | |
| 165 | 170 | 175 | |
| Tyr Lys Asp Tyr Ile Arg Glu Arg Phe Asp Asn Glu Thr Phe Arg Ile | | | |
| 180 | 185 | 190 | |
| Ser Val Tyr Gln Val Leu Gln Glu His Leu Gly Arg Glu Ser Asp Leu | | | |
| 195 | 200 | 205 | |

- 56 -

Phe Leu Leu Asp Ser Arg Thr Leu Trp Ala Ser Glu Glu Gly Trp Leu
210 215 220

Val Phe Asp Ile Thr Ala Thr Ser Asn His Trp Val Val Asn Pro Arg
225 230 235 240

His Asn Leu Gly Leu Gln Leu Ser Val Glu Thr Leu Asp Gly Gln Ser
245 250 255

Ile Asn Pro Lys Leu Ala Gly Leu Ile Gly Arg His Gly Pro Gln Asn
260 265 270

Lys Gln Pro Phe Met Val Ala Phe Phe Lys Ala Thr Glu Val His Phe
275 280 285

Arg Ser Ile Arg Ser Thr Gly Ser Lys Gln Arg Ser Gln Asn Arg Ser
290 295 300

Lys Thr Pro Lys Asn Gln Glu Ala Leu Arg Met Ala Asn Val Ala Glu
305 310 315 320

Asn Ser Ser Ser Asp Gln Arg Gln Ala Cys Lys Lys His Glu Leu Tyr
325 330 335

Val Ser Phe Arg Asp Leu Gly Trp Gln Asp Trp Ile Ile Ala Pro Glu
340 345 350

Gly Tyr Ala Ala Tyr Tyr Cys Glu Gly Glu Cys Ala Phe Pro Leu Asn
355 360 365

Ser Tyr Met Asn Ala Thr Asn His Ala Ile Val Gln Thr Leu Val His
370 375 380

Phe Ile Asn Pro Glu Thr Val Pro Lys Pro Cys Cys Ala Pro Thr Gln
385 390 395 400

Leu Asn Ala Ile Ser Val Leu Tyr Phe Asp Asp Ser Ser Asn Val Ile
405 410 415

Leu Lys Lys Tyr Arg Asn Met Val Val Arg Ala Cys Gly Cys His
420 425 430

(2) INFORMATION FOR SEQ ID NO:17:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1873 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

- 57 -

(vi) ORIGINAL SOURCE:

(A) ORGANISM: MURIDAE
 (F) TISSUE TYPE: EMBRYO

(ix) FEATURE:

(A) NAME/KEY: CDS
 (B) LOCATION: 104..1393
 (D) OTHER INFORMATION: /function= "OSTEOGENIC PROTEIN"
 /product= "MOP1"
 /note= "MOP1 (CDNA)"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

| | | | | | | |
|-------------------------|---------------------|-------------------------|-------------|---------------------|------------|-----|
| CTGCAGCAAG | TGACCTCGGG | TCGTGGACCG | CTGCCCTGCC | CCCTCCGCTG | CCACCTGGGG | 60 |
| CGGCGCGGGC | CCGGTGCCCC | GGATCGCGCG | TAGAGCCGGC | GCG ATG CAC GTG CGC | | 115 |
| | | | | Met His Val Arg | | |
| | | | | 1 | | |
| TCG CTG CGC GCT GCG GCG | CCA CAC AGC TTC | GTG GCG CTC | TGG GCG CCT | | | 163 |
| Ser Leu Arg Ala Ala Ala | Pro His Ser Phe | Val Ala Leu Trp | Ala Pro | | | |
| 5 | 10 | 15 | 20 | | | |
| CTG TTC TTG CTG CGC TCC | GCC CTG GCC GAT | TTC AGC CTG GAC | AAC AAC GAG | | | 211 |
| Leu Phe Leu Leu Arg Ser | Ala Leu Ala Asp | Phe Ser Leu Asp | Asn Glu | | | |
| 25 | 30 | 35 | | | | |
| GTG CAC TCC AGC TTC ATC | CAC CGG CGC CTC | CGC AGC CAG GAG | CGG CGG | | | 259 |
| Val His Ser Ser Phe Ile | His Arg Arg Leu | Arg Ser Gln Glu | Arg Arg | | | |
| 40 | 45 | 50 | | | | |
| GAG ATG CAG CGG GAG ATC | CTG TCC ATC TTA | GGG TTG CCC CAT CGC CCG | | | | 307 |
| Glu Met Gln Arg Glu Ile | Leu Ser Ile Leu | Gly Leu Pro His | Arg Pro | | | |
| 55 | 60 | 65 | | | | |
| CGC CCG CAC CTC CAG GGA | AAG CAT AAT TCG | GCG CCC ATG TTC ATG TTG | | | | 355 |
| Arg Pro His Leu Gln Gly | Lys His Asn Ser | Ala Pro Met Phe | Met Leu | | | |
| 70 | 75 | 80 | | | | |
| GAC CTG TAC AAC GCC ATG | GCG GTG GAG GAG | AGC GGG CCG GAC GGA CAG | | | | 403 |
| Asp Leu Tyr Asn Ala Met | Ala Val Glu Glu | Ser Gly Pro Asp Gly | Gln | | | |
| 85 | 90 | 95 | 100 | | | |
| GGC TTC TCC TAC CCC TAC | AAG GCC GTC TTC | AGT ACC CAG GGC CCC CCT | | | | 451 |
| Gly Phe Ser Tyr Pro Tyr | Lys Ala Val Phe | Ser Thr Gln Gly Pro | Pro Pro | | | |
| 105 | 110 | 115 | | | | |
| TTA GCC AGC CTG CAG GAC | CAT TTC CTC ACT GAC | GCC GAC ATG GTC | | | | 499 |
| Leu Ala Ser Leu Gln Asp | Ser His Phe Leu | Thr Asp Ala Asp | Met Val | | | |
| 120 | 125 | 130 | | | | |
| ATG AGC TTC GTC AAC CTA | GTG GAA CAT GAC AAA | GAA TTC TTC CAC CCT | | | | 547 |
| Met Ser Phe Val Asn Leu | Val Glu His Asp Lys | Glu Phe Phe His | Pro | | | |
| 135 | 140 | 145 | | | | |
| CGA TAC CAC CAT CGG GAG | TTC CGG TTT GAT | CTT TCC AAG ATC CCC GAG | | | | 595 |

- 58 -

| | | | |
|---|-----|-----|------|
| Arg Tyr His His Arg Glu Phe Arg Phe Asp Leu Ser Lys Ile Pro Glu | | | |
| 150 | 155 | 160 | |
| GGC GAA CGG GTG ACC GCA GCC GAA TTC AGG ATC TAT AAG GAC TAC ATC | | | 643 |
| Gly Glu Arg Val Thr Ala Ala Glu Phe Arg Ile Tyr Lys Asp Tyr Ile | | | |
| 165 | 170 | 175 | 180 |
| CGG GAG CGA TTT GAC AAC GAG ACC TTC CAG ATC ACA GTC TAT CAG GTG | | | 691 |
| Arg Glu Arg Phe Asp Asn Glu Thr Phe Gln Ile Thr Val Tyr Gln Val | | | |
| 185 | 190 | 195 | |
| CTC CAG GAG CAC TCA GGC AGG GAG TCG GAC CTC TTC TTG CTG GAC AGC | | | 739 |
| Leu Gln Glu His Ser Gly Arg Glu Ser Asp Leu Phe Leu Leu Asp Ser | | | |
| 200 | 205 | 210 | |
| CGC ACC ATC TGG GCT TCT GAG GAG GGC TGG TTG GTG TTT GAT ATC ACA | | | 787 |
| Arg Thr Ile Trp Ala Ser Glu Glu Gly Trp Leu Val Phe Asp Ile Thr | | | |
| 215 | 220 | 225 | |
| GCC ACC AGC AAC CAC TGG GTG GTC AAC CCT CGG CAC AAC CTG GGC TTA | | | 835 |
| Ala Thr Ser Asn His Trp Val Val Asn Pro Arg His Asn Leu Gly Leu | | | |
| 230 | 235 | 240 | |
| CAG CTC TCT GTG GAG ACC CTG GAT GGG CAG AGC ATC AAC CCC AAG TTG | | | 883 |
| Gln Leu Ser Val Glu Thr Leu Asp Gly Gln Ser Ile Asn Pro Lys Leu | | | |
| 245 | 250 | 255 | 260 |
| GCA GGC CTG ATT GGA CGG CAT GGA CCC CAG AAC AAG CAA CCC TTC ATG | | | 931 |
| Ala Gly Leu Ile Gly Arg His Gly Pro Gln Asn Lys Gln Pro Phe Met | | | |
| 265 | 270 | 275 | |
| GTG GCC TTC TTC AAG GCC ACG GAA GTC CAT CTC CGT AGT ATC CGG TCC | | | 979 |
| Val Ala Phe Phe Lys Ala Thr Glu Val His Leu Arg Ser Ile Arg Ser | | | |
| 280 | 285 | 290 | |
| ACG GGG GGC AAG CAG CGC AGC CAG AAT CGC TCC AAG ACG CCA AAG AAC | | | 1027 |
| Thr Gly Lys Gln Arg Ser Gln Asn Arg Ser Lys Thr Pro Lys Asn | | | |
| 295 | 300 | 305 | |
| CAA GAG GCC CTG AGG ATG GCC AGT GTG GCA GAA AAC AGC AGC AGT GAC | | | 1075 |
| Gln Glu Ala Leu Arg Met Ala Ser Val Ala Glu Asn Ser Ser Ser Asp | | | |
| 310 | 315 | 320 | |
| CAG AGG CAG GCC TGC AAG AAA CAT GAG CTG TAC GTC AGC AGC TTC CGA GAC | | | 1123 |
| Gln Arg Gln Ala Cys Lys Lys His Glu Leu Tyr Val Ser Phe Arg Asp | | | |
| 325 | 330 | 335 | 340 |
| CTT GGC TGG CAG GAC TGG ATC ATT GCA CCT GAA GGC TAT GCT GCC TAC | | | 1171 |
| Leu Gly Trp Gln Asp Trp Ile Ile Ala Pro Glu Gly Tyr Ala Ala Tyr | | | |
| 345 | 350 | 355 | |
| TAC TGT GAG GGA GAG TGC GCC TTC CCT CTG AAC TCC TAC ATG AAC GCC | | | 1219 |
| Tyr Cys Glu Gly Glu Cys Ala Phe Pro Leu Asn Ser Tyr Met Asn Ala | | | |
| 360 | 365 | 370 | |
| ACC AAC CAC GCC ATC GTC CAG ACA CTG GTT CAC TTC ATC AAC CCA GAC | | | 1267 |
| Thr Asn His Ala Ile Val Gln Thr Leu Val His Phe Ile Asn Pro Asp | | | |

- 59 -

375

380

385

| | |
|---|------|
| ACA GTA CCC AAG CCC TGC TGT GCG CCC ACC CAG CTC AAC GCC ATC TCT | 1315 |
| Thr Val Pro Lys Pro Cys Cys Ala Pro Thr Gln Leu Asn Ala Ile Ser | |
| 390 395 400 | |
| GTC CTC TAC TTC GAC GAC AGC TCT AAT GTC ATC CTG AAG AAG TAC AGA | 1363 |
| Val Leu Tyr Phe Asp Asp Ser Ser Asn Val Ile Leu Lys Lys Tyr Arg | |
| 405 410 415 420 | |
| AAC ATG GTG GTC CGG GCC TGT GGC TGC CAC TAGCTTTCC TGAGACCCTG | 1413 |
| Asn Met Val Val Arg Ala Cys Gly Cys His | |
| 425 430 | |
| ACCTTGCGG GGCCACACCT TTCCAAATCT TCGATGTCTC ACCATCTAAG TCTCTCACTG | 1473 |
| CCCACCTTGG CGAGGAGAAC AGACCAAACCT CTCCTGAGCC TTCCCTCACCC TCCCAACCGG | 1533 |
| AAGCATGTAA GGGTCCAGA AACCTGAGCG TGCAGCAGCT GATGAGCGCC CTTTCCTTCT | 1593 |
| GGCACGTGAC GGACAAGATC CTACCAGCTA CCACAGCAAA CGCCTAAGAG CAGGAAAAAT | 1653 |
| GTCTGCCAGG AAAGTGTCCA GTGTCCACAT GGCCCCCTGGC GCTCTGAGTC TTTGAGGAGT | 1713 |
| AATCGCAAGC CTCGTTCAAGC TGCAGCAGAA GGAAGGGCTT AGCCAGGGTG GGCGCTGGCG | 1773 |
| TCTGTGTTGA AGGGAAACCA AGCAGAAGCC ACTGTAATGA TATGTCACAA TAAAACCCAT | 1833 |
| GAATGAAAAA AAAAAAAAAA AAAAAAAAAA AAAAGAATTG | 1873 |

(2) INFORMATION FOR SEQ ID NO:18:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 430 amino acids
 - (B) TYPE: amino acid
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

Met His Val Arg Ser Leu Arg Ala Ala Ala Pro His Ser Phe Val Ala
1 5 10 15

Leu Trp Ala Pro Leu Phe Leu Leu Arg Ser Ala Leu Ala Asp Phe Ser
20 25 30

Leu Asp Asn Glu Val His Ser Ser Phe Ile His Arg Arg Leu Arg Ser
35 40 45

Gln Glu Arg Arg Glu Met Gln Arg Glu Ile Leu Ser Ile Leu Gly Leu
50 55 60

Pro His Arg Pro Arg Pro His Leu Gln Gly Lys His Asn Ser Ala Pro
65 70 75 80

- 60 -

Met Phe Met Leu Asp Leu Tyr Asn Ala Met Ala Val Glu Glu Ser Gly
85 90 95

Pro Asp Gly Gln Gly Phe Ser Tyr Pro Tyr Lys Ala Val Phe Ser Thr
100 105 110

Gln Gly Pro Pro Leu Ala Ser Leu Gln Asp Ser His Phe Leu Thr Asp
115 120 125

Ala Asp Met Val Met Ser Phe Val Asn Leu Val Glu His Asp Lys Glu
130 135 140

Phe Phe His Pro Arg Tyr His His Arg Glu Phe Arg Phe Asp Leu Ser
145 150 155 160

Lys Ile Pro Glu Gly Glu Arg Val Thr Ala Ala Glu Phe Arg Ile Tyr
165 170 175

Lys Asp Tyr Ile Arg Glu Arg Phe Asp Asn Glu Thr Phe Gln Ile Thr
180 185 190

Val Tyr Gln Val Leu Gln Glu His Ser Gly Arg Glu Ser Asp Leu Phe
195 200 205

Leu Leu Asp Ser Arg Thr Ile Trp Ala Ser Glu Glu Gly Trp Leu Val
210 215 220

Phe Asp Ile Thr Ala Thr Ser Asn His Trp Val Val Asn Pro Arg His
225 230 235 240

Asn Leu Gly Leu Gln Leu Ser Val Glu Thr Leu Asp Gly Gln Ser Ile
245 250 255

Asn Pro Lys Leu Ala Gly Leu Ile Gly Arg His Gly Pro Gln Asn Lys
260 265 270

Gln Pro Phe Met Val Ala Phe Phe Lys Ala Thr Glu Val His Leu Arg
275 280 285

Ser Ile Arg Ser Thr Gly Gly Lys Gln Arg Ser Gln Asn Arg Ser Lys
290 295 300

Thr Pro Lys Asn Gln Glu Ala Leu Arg Met Ala Ser Val Ala Glu Asn
305 310 315 320

Ser Ser Ser Asp Gln Arg Gln Ala Cys Lys Lys His Glu Leu Tyr Val
325 330 335

Ser Phe Arg Asp Leu Gly Trp Gln Asp Trp Ile Ile Ala Pro Glu Gly
340 345 350

Tyr Ala Ala Tyr Tyr Cys Glu Gly Glu Cys Ala Phe Pro Leu Asn Ser
355 360 365

Tyr Met Asn Ala Thr Asn His Ala Ile Val Gln Thr Leu Val His Phe
370 375 380

- 61 -

Ile Asn Pro Asp Thr Val Pro Lys Pro Cys Cys Ala Pro Thr Gln Leu
 385 390 395 400

Asn Ala Ile Ser Val Leu Tyr Phe Asp Asp Ser Ser Asn Val Ile Leu
 405 410 415

Lys Lys Tyr Arg Asn Met Val Val Arg Ala Cys Gly Cys His
 420 425 430

(2) INFORMATION FOR SEQ ID NO:19:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1723 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: Homo sapiens
- (F) TISSUE TYPE: HIPPOCAMPUS

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 490..1696
- (D) OTHER INFORMATION: /function= "OSTEOGENIC PROTEIN"
 /product= "hOP2-PP"
 /note= "hOP2 (cDNA)"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

GGCGCCGGCA GAGCAGGAGT GGCTGGAGGA GCTGTGGTTG GAGCAGGAGG TGGCACGGCA 60

GGGCTGGAGG GCTCCCTATG AGTGGCGGAG ACGGCCAGG AGGCGCTGGA GCAACAGCTC 120

CCACACCGCA CCAAGCGGTG GCTGCAGGAG CTGGCCCATC GCCCCCTGCGC TGCTCGGACC 180

GCGGCCACAG CGGGACTGGC GGGTACGGCG GCGACAGAGG CATTGGCCGA GAGTCCCAGT 240

CCGCAGAGTA GCCCCGGCCT CGAGGCCGTG GCGTCCCGGT CCTCTCCGTC CAGGAGCCAG 300

GACAGGTGTC GCGCGGGGG GCTCCAGGGA CCGCGCCTGA GGCCGGCTGC CCGCCCGTCC 360

CGCCCCGCCC CGCCGCCCGA GCCCAGCTC CTTGCCGTGC GGGCGTCCCC 420

AGGCCCTGGG TCGGCCGCGG AGCCGATGCG CGCCCGCTGA GCGCCCCAGC TGAGCGCCCC 480

CGGCCTGCC ATG ACC GCG CTC CCC GGC CCG CTC TGG CTC CTG GGC CTG 528
 Met Thr Ala Leu Pro Gly Pro Leu Trp Leu Leu Gly Leu
 1 5 10

GCG CTA TGC GCG CTG GGC GGG GGC GGC CCC GGC CTG CGA CCC CCG CCC
 Ala Leu Cys Ala Leu Gly Gly Gly Pro Gly Leu Arg Pro Pro Pro 576
 15 20 25

- 62 -

| | |
|---|------|
| GGC TGT CCC CAG CGA CGT CTG GGC GCG CGC GAG CGC CGG GAC GTG CAG Gly Cys Pro Gln Arg Arg Leu Gly Ala Arg Glu Arg Arg Asp Val Gln 30 35 40 45 | 624 |
| CGC GAG ATC CTG GCG GTG CTC GGG CTG CCT GGG CGG CCC CGG CCC CGC Arg Glu Ile Leu Ala Val Leu Gly Leu Pro Gly Arg Pro Arg Pro Arg 50 55 60 | 672 |
| GCG CCA CCC GCC GCC TCC CGG CTG CCC GCG TCC GCG CCG CTC TTC ATG Ala Pro Pro Ala Ala Ser Arg Leu Pro Ala Ser Ala Pro Leu Phe Met 65 70 75 | 720 |
| CTG GAC CTG TAC CAC GCC ATG GCC GGC GAC GAC GAG GAC GGC GCG Leu Asp Leu Tyr His Ala Met Ala Gly Asp Asp Asp Glu Asp Gly Ala 80 85 90 | 768 |
| CCC GCG GAG CGG CGC CTG GGC CGC GCC GAC CTG GTC ATG AGC TTC GTT Pro Ala Glu Arg Arg Leu Gly Arg Ala Asp Leu Val Met Ser Phe Val 95 100 105 | 816 |
| AAC ATG GTG GAG CGA GAC CGT GCC CTG GGC CAC CAG GAG CCC CAT TGG Asn Met Val Glu Arg Asp Arg Ala Leu Gly His Gln Glu Pro His Trp 110 115 120 125 | 864 |
| AAG GAG TTC CGC TTT GAC CTG ACC CAG ATC CCG GCT GGG GAG GCG GTC Lys Glu Phe Arg Phe Asp Leu Thr Gln Ile Pro Ala Gly Glu Ala Val 130 135 140 | 912 |
| ACA GCT GCG GAG TTC CGG ATT TAC AAG GTG CCC AGC ATC CAC CTG CTC Thr Ala Ala Glu Phe Arg Ile Tyr Lys Val Pro Ser Ile His Leu Leu 145 150 155 | 960 |
| AAC AGG ACC CTC CAC GTC AGC ATG TTC CAG GTG GTC CAG GAG CAG TCC Asn Arg Thr Leu His Val Ser Met Phe Gln Val Val Gln Glu Gln Ser 160 165 170 | 1008 |
| AAC AGG GAG TCT GAC TTG TTC TTT TTG GAT CTT CAG ACG CTC CGA GCT Asn Arg Glu Ser Asp Leu Phe Phe Leu Asp Leu Gln Thr Leu Arg Ala 175 180 185 | 1056 |
| GGA GAC GAG GGC TGG CTG GTG CTG GAT GTC ACA GCA GCC AGT GAC TGC Gly Asp Glu Gly Trp Leu Val Leu Asp Val Thr Ala Ala Ser Asp Cys 190 195 200 205 | 1104 |
| TGG TTG CTG AAG CGT CAC AAG GAC CTG GGA CTC CGC CTC TAT GTG GAG Trp Leu Leu Lys Arg His Lys Asp Leu Gly Leu Arg Leu Tyr Val Glu 210 215 220 | 1152 |
| ACT GAG GAC GGG CAC AGC GTG GAT CCT GGC CTG GCC GGC CTG CTG GGT Thr Glu Asp Gly His Ser Val Asp Pro Gly Leu Ala Gly Leu Leu Gly 225 230 235 | 1200 |
| CAA CGG GCC CCA CGC TCC CAA CAG CCT TTC GTG GTC ACT TTC TTC AGG Gln Arg Ala Pro Arg Ser Gln Gln Pro Phe Val Val Thr Phe Phe Arg 240 245 250 | 1248 |
| GCC AGT CCG AGT CCC ATC CGC ACC CCT CGG GCA GTG AGG CCA CTG AGG | 1296 |

- 63 -

| | | | |
|---|-----|-----|------|
| Ala Ser Pro Ser Pro Ile Arg Thr Pro Arg Ala Val Arg Pro Leu Arg | | | |
| 255 | 260 | 265 | |
| AGG AGG CAG CCG AAG AAA AGC AAC GAG CTG CCG CAG GCC AAC CGA CTC | | | 1344 |
| Arg Arg Gln Pro Lys Lys Ser Asn Glu Leu Pro Gln Ala Asn Arg Leu | | | |
| 270 | 275 | 280 | 285 |
| CCA GGG ATC TTT GAT GAC GTC CAC GGC TCC CAC GGC CGG CAG GTC TGC | | | 1392 |
| Pro Gly Ile Phe Asp Asp Val His Gly Ser His Gly Arg Gln Val Cys | | | |
| 290 | 295 | 300 | |
| CGT CGG CAC GAG CTC TAC GTC AGC TTC CAG GAC CTC GGC TGG CTG GAC | | | 1440 |
| Arg Arg His Glu Leu Tyr Val Ser Phe Gln Asp Leu Gly Trp Leu Asp | | | |
| 305 | 310 | 315 | |
| TGG GTC ATC GCT CCC CAA GGC TAC TCG GCC TAT TAC TGT GAG GGG GAG | | | 1488 |
| Trp Val Ile Ala Pro Gln Gly Tyr Ser Ala Tyr Tyr Cys Glu Gly Glu | | | |
| 320 | 325 | 330 | |
| TGC TCC TTC CCA CTG GAC TCC TGC ATG AAT GCC ACC AAC CAC GCC ATC | | | 1536 |
| Cys Ser Phe Pro Leu Asp Ser Cys Met Asn Ala Thr Asn His Ala Ile | | | |
| 335 | 340 | 345 | |
| CTG CAG TCC CTG GTG CAC CTG ATG AAG CCA AAC GCA GTC CCC AAG GCG | | | 1584 |
| Leu Gln Ser Leu Val His Leu Met Lys Pro Asn Ala Val Pro Lys Ala | | | |
| 350 | 355 | 360 | 365 |
| TGC TGT GCA CCC ACC AAG CTG AGC GCC ACC TCT GTG CTC TAC TAT GAC | | | 1632 |
| Cys Cys Ala Pro Thr Lys Leu Ser Ala Thr Ser Val Leu Tyr Tyr Asp | | | |
| 370 | 375 | 380 | |
| AGC AGC AAC AAC GTC ATC CTG CGC AAA CAC CGC AAC ATG GTG GTC AAG | | | 1680 |
| Ser Ser Asn Asn Val Ile Leu Arg Lys His Arg Asn Met Val Val Lys | | | |
| 385 | 390 | 395 | |
| GCC TGC GGC TGC CAC T GAGTCAGCCC GCCCAGCCCT ACTGCAG | | | 1723 |
| Ala Cys Gly Cys His | | | |
| 400 | | | |

(2) INFORMATION FOR SEQ ID NO:20:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 402 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

| | | | |
|---|----|----|----|
| Met Thr Ala Leu Pro Gly Pro Leu Trp Leu Leu Gly Leu Ala Leu Cys | | | |
| 1 | 5 | 10 | 15 |
| Ala Leu Gly Gly Gly Pro Gly Leu Arg Pro Pro Pro Gly Cys Pro | | | |
| 20 | 25 | 30 | |

- 64 -

Gln Arg Arg Leu Gly Ala Arg Glu Arg Asp Val Gln Arg Glu Ile
35 40 45

Leu Ala Val Leu Gly Leu Pro Gly Arg Pro Arg Pro Arg Ala Pro Pro
50 55 60

Ala Ala Ser Arg Leu Pro Ala Ser Ala Pro Leu Phe Met Leu Asp Leu
65 70 75 80

Tyr His Ala Met Ala Gly Asp Asp Asp Glu Asp Gly Ala Pro Ala Glu
85 90 95

Arg Arg Leu Gly Arg Ala Asp Leu Val Met Ser Phe Val Asn Met Val
100 105 110

Glu Arg Asp Arg Ala Leu Gly His Gln Glu Pro His Trp Lys Glu Phe
115 120 125

Arg Phe Asp Leu Thr Gln Ile Pro Ala Gly Glu Ala Val Thr Ala Ala
130 135 140

Glu Phe Arg Ile Tyr Lys Val Pro Ser Ile His Leu Leu Asn Arg Thr
145 150 155 160

Leu His Val Ser Met Phe Gln Val Val Gln Glu Gln Ser Asn Arg Glu
165 170 175

Ser Asp Leu Phe Phe Leu Asp Leu Gln Thr Leu Arg Ala Gly Asp Glu
180 185 190

Gly Trp Leu Val Leu Asp Val Thr Ala Ala Ser Asp Cys Trp Leu Leu
195 200 205

Lys Arg His Lys Asp Leu Gly Leu Arg Leu Tyr Val Glu Thr Glu Asp
210 215 220

Gly His Ser Val Asp Pro Gly Leu Ala Gly Leu Leu Gly Gln Arg Ala
225 230 235 240

Pro Arg Ser Gln Gln Pro Phe Val Val Thr Phe Phe Arg Ala Ser Pro
245 250 255

Ser Pro Ile Arg Thr Pro Arg Ala Val Arg Pro Leu Arg Arg Arg Gln
260 265 270

Pro Lys Lys Ser Asn Glu Leu Pro Gln Ala Asn Arg Leu Pro Gly Ile
275 280 285

Phe Asp Asp Val His Gly Ser His Gly Arg Gln Val Cys Arg Arg His
290 295 300

Glu Leu Tyr Val Ser Phe Gln Asp Leu Gly Trp Leu Asp Trp Val Ile
305 310 315 320

Ala Pro Gln Gly Tyr Ser Ala Tyr Tyr Cys Glu Gly Glu Cys Ser Phe
325 330 335

- 65 -

Pro Leu Asp Ser Cys Met Asn Ala Thr Asn His Ala Ile Leu Gln Ser
340 345 350

Leu Val His Leu Met Lys Pro Asn Ala Val Pro Lys Ala Cys Cys Ala
355 360 365

Pro Thr Lys Leu Ser Ala Thr Ser Val Leu Tyr Tyr Asp Ser Ser Asn
370 375 380

Asn Val Ile Leu Arg Lys His Arg Asn Met Val Val Lys Ala Cys Gly
 385 390 395 400

Cys His

(2) INFORMATION FOR SEQ ID NO:21:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1926 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: MURIDAE
(F) TISSUE TYPE: EMBRYO

(ix) FEATURE:

- (A) NAME/KEY: CDS
(B) LOCATION: 93..1289
(D) OTHER INFORMATION: /function= "OSTEOGENIC PROTEIN"
 /product= "mOP2-PP"
 /note= "mOP2 cDNA"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

GCCAGGCACA GGTGCGCCGT CTGGTCCCTCC CCGTCTGGCG TCAGGCCGAGC CCGACCAGCT 60

ACCAAGTGGAT GCGCGCCGGC TGAAAGTCCG AG ATG GCT ATG CGT CCC GGG CCA 113
Met Ala Met Arg Pro Gly Pro
1 5

CCG CGT CCC CCG CAC ACC TGT CCC CAG CGT CGC CTG GGA GCG CGC GAG
 Pro Arg Pro Pro His Thr Cys Pro Gln Arg Arg Leu Gly Ala Arg Glu
 25 30 35

CGC CGC GAC ATG CAG CGT GAA ATC CTG GCG GTG CTC GGG CTA CCG GGA 257
 Arg Arg Asp Met Gln Arg Glu Ile Leu Ala Val Leu Gly Leu Pro Gly
 40 45 50 55

CGG CCC CGA CCC CGT GCA CAA CCC GCC GCT GCC CGG CAG CCA GCG TCC 305
Arg Pro Arg Pro Arg Ala Gln Pro Ala Ala Ala Arg Gln Pro Ala Ser

- 66 -

| 60 | 65 | 70 | |
|---|----|----|-----|
| GCG CCC CTC TTC ATG TTG GAC CTA TAC CAC GCC ATG ACC GAT GAC GAC Ala Pro Leu Phe Met Leu Asp Leu Tyr His Ala Met Thr Asp Asp Asp 75 80 85 | | | 353 |
| GAC GGC GGG CCA CCA CAG GCT CAC TTA GGC CGT GCC GAC CTG GTC ATG Asp Gly Gly Pro Pro Gln Ala His Leu Gly Arg Ala Asp Leu Val Met 90 95 100 | | | 401 |
| AGC TTC GTC AAC ATG GTG GAA CGC GAC CGT ACC CTG GGC TAC CAG GAG Ser Phe Val Asn Met Val Glu Arg Asp Arg Thr Leu Gly Tyr Gln Glu 105 110 115 | | | 449 |
| CCA CAC TGG AAG GAA TTC CAC TTT GAC CTA ACC CAG ATC CCT GCT GGG Pro His Trp Lys Glu Phe His Phe Asp Leu Thr Gln Ile Pro Ala Gly 120 125 130 135 | | | 497 |
| GAG GCT GTC ACA GCT GCT GAG TTC CGG ATC TAC AAA GAA CCC AGC ACC Glu Ala Val Thr Ala Ala Glu Phe Arg Ile Tyr Lys Glu Pro Ser Thr 140 145 150 | | | 545 |
| CAC CCG CTC AAC ACA ACC CTC CAC ATC AGC ATG TTC GAA GTG GTC CAA His Pro Leu Asn Thr Thr Leu His Ile Ser Met Phe Glu Val Val Gln 155 160 165 | | | 593 |
| GAG CAC TCC AAC AGG GAG TCT GAC TTG TTC TTT TTG GAT CTT CAG ACG Glu His Ser Asn Arg Glu Ser Asp Leu Phe Phe Leu Asp Leu Gln Thr 170 175 180 | | | 641 |
| CTC CGA TCT GGG GAC GAG GGC TGG CTG GTG CTG GAC ATC ACA GCA GCC Leu Arg Ser Gly Asp Glu Gly Trp Leu Val Leu Asp Ile Thr Ala Ala 185 190 195 | | | 689 |
| AGT GAC CGA TGG CTG CTG AAC CAT CAC AAG GAC CTG GGA CTC CGC CTC Ser Asp Arg Trp Leu Leu Asn His His Lys Asp Leu Gly Leu Arg Leu 200 205 210 215 | | | 737 |
| TAT GTG GAA ACC GCG GAT GGG CAC AGC ATG GAT CCT GGC CTG GCT GGT Tyr Val Glu Thr Ala Asp Gly His Ser Met Asp Pro Gly Leu Ala Gly 220 225 230 | | | 785 |
| CTG CTT GGA CGA CAA GCA CCA CGC TCC AGA CAG CCT TTC ATG GTA ACC Leu Leu Gly Arg Gln Ala Pro Arg Ser Arg Gln Pro Phe Met Val Thr 235 240 245 | | | 833 |
| TTC TTC AGG GCC AGC CAG AGT CCT GTG CGG GCC CCT CGG GCA GCG AGA Phe Phe Arg Ala Ser Gln Ser Pro Val Arg Ala Pro Arg Ala Ala Arg 250 255 260 | | | 881 |
| CCA CTG AAG AGG AGG CAG CCA AAG AAA ACG AAC GAG CTT CCG CAC CCC Pro Leu Lys Arg Arg Gln Pro Lys Lys Thr Asn Glu Leu Pro His Pro 265 270 275 | | | 929 |
| AAC AAA CTC CCA GGG ATC TTT GAT GAT GGC CAC GGT TCC CGC GGC AGA Asn Lys Leu Pro Gly Ile Phe Asp Asp Gly His Gly Ser Arg Gly Arg 280 285 290 295 | | | 977 |

- 67 -

| | |
|---|------|
| GAG GTT TGC CGC AGG CAT GAG CTC TAC GTC AGC TTC CGT GAC CTT GGC Glu Val Cys Arg Arg His Glu Leu Tyr Val Ser Phe Arg Asp Leu Gly 300 305 310 | 1025 |
| TGG CTG GAC TGG GTC ATC GCC CCC CAG GGC TAC TCT GCC TAT TAC TGT Trp Leu Asp Trp Val Ile Ala Pro Gln Gly Tyr Ser Ala Tyr Tyr Cys 315 320 325 | 1073 |
| GAG GGG GAG TGT GCT TTC CCA CTG GAC TCC TGT ATG AAC GCC ACC AAC Glu Gly Glu Cys Ala Phe Pro Leu Asp Ser Cys Met Asn Ala Thr Asn 330 335 340 | 1121 |
| CAT GCC ATC TTG CAG TCT CTG GTG CAC CTG ATG AAG CCA GAT GTT GTC His Ala Ile Leu Gln Ser Leu Val His Leu Met Lys Pro Asp Val Val 345 350 355 | 1169 |
| CCC AAG GCA TGC TGT GCA CCC ACC AAA CTG AGT GCC ACC TCT GTG CTG Pro Lys Ala Cys Cys Ala Pro Thr Lys Leu Ser Ala Thr Ser Val Leu 360 365 370 375 | 1217 |
| TAC TAT GAC AGC AGC AAC AAT GTC ATC CTG CGT AAA CAC CGT AAC ATG Tyr Tyr Asp Ser Ser Asn Asn Val Ile Leu Arg Lys His Arg Asn Met 380 385 390 | 1265 |
| GTC GTC AAG GCC TGT GGC TGC CAC TGAGGCCCG CCCAGCATCC TGCTTCTACT Val Val Lys Ala Cys Gly Cys His 395 | 1319 |
| ACCTTACCAT CTGGCCGGGC CCCTCTCCAG AGGCAGAAAC CCTTCTATGT TATCATAGCT | 1379 |
| CAGACAGGGG CAATGGGAGG CCCTTCACTT CCCCTGGCCA CTTCTGCTA AAATTCTGGT | 1439 |
| CTTTCCCAGT TCCTCTGTCC TTCATGGGT TTGGGGCTA TCACCCCGCC CTCTCCATCC | 1499 |
| TCCTACCCCA AGCATAGACT GAATGCACAC ACCATCCCAG AGCTATGCTA ACTGAGAGGT | 1559 |
| CTGGGGTCAG CACTGAAGGC CCACATGAGG AAGACTGATC CTTGGCCATC CTCAGCCAC | 1619 |
| AATGGCAAAT TCTGGATGGT CTAAGAAGGC CCTGGAATTCAAAACTAGAT GATCTGGCT | 1679 |
| CTCTGCACCA TTCTTGTTGG CAGTTGGGAC ATTTTAGGT ATAACAGACA CATAACTTA | 1739 |
| GATCAATGCA TCGCTGTACT CCTTGAAATC AGAGCTAGCT TGTTAGAAAA AGAACAGAG | 1799 |
| CCAGGTATAG CGGTGCATGT CATTAAATCCC AGCGCTAAAG AGACAGAGAC AGGAGAACT | 1859 |
| CTGTGAGTTC AAGGCCACAT AGAAAGAGCC TGTCTCGGGA GCAGGAAAAA AAAAAAAAC | 1919 |
| GGAATTC | 1926 |

(2) INFORMATION FOR SEQ ID NO:22:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 399 amino acids
- (B) TYPE: amino acid

- 68 -

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

Met Ala Met Arg Pro Gly Pro Leu Trp Leu Leu Gly Leu Ala Leu Cys
1 5 10 15

Ala Leu Gly Gly Gly His Gly Pro Arg Pro Pro His Thr Cys Pro Gln
20 25 30

Arg Arg Leu Gly Ala Arg Glu Arg Arg Asp Met Gln Arg Glu Ile Leu
35 40 45

Ala Val Leu Gly Leu Pro Gly Arg Pro Arg Pro Arg Ala Gln Pro Ala
50 55 60

Ala Ala Arg Gln Pro Ala Ser Ala Pro Leu Phe Met Leu Asp Leu Tyr
65 70 75 80

His Ala Met Thr Asp Asp Asp Gly Gly Pro Pro Gln Ala His Leu
85 90 95

Gly Arg Ala Asp Leu Val Met Ser Phe Val Asn Met Val Glu Arg Asp
100 105 110

Arg Thr Leu Gly Tyr Gln Glu Pro His Trp Lys Glu Phe His Phe Asp
115 120 125

Leu Thr Gln Ile Pro Ala Gly Glu Ala Val Thr Ala Ala Glu Phe Arg
130 135 140

Ile Tyr Lys Glu Pro Ser Thr His Pro Leu Asn Thr Thr Leu His Ile
145 150 155 160

Ser Met Phe Glu Val Val Gln Glu His Ser Asn Arg Glu Ser Asp Leu
165 170 175

Phe Phe Leu Asp Leu Gln Thr Leu Arg Ser Gly Asp Glu Gly Trp Leu
180 185 190

Val Leu Asp Ile Thr Ala Ala Ser Asp Arg Trp Leu Leu Asn His His
195 200 205

Lys Asp Leu Gly Leu Arg Leu Tyr Val Glu Thr Ala Asp Gly His Ser
210 215 220

Met Asp Pro Gly Leu Ala Gly Leu Leu Gly Arg Gln Ala Pro Arg Ser
225 230 235 240

Arg Gln Pro Phe Met Val Thr Phe Phe Arg Ala Ser Gln Ser Pro Val
245 250 255

Arg Ala Pro Arg Ala Ala Arg Pro Leu Lys Arg Arg Gln Pro Lys Lys
260 265 270

- 69 -

| | | | |
|---|-----|-----|-----|
| Thr Asn Glu Leu Pro His Pro Asn Lys Leu Pro Gly Ile Phe Asp Asp | | | |
| 275 | 280 | 285 | |
| Gly His Gly Ser Arg Gly Arg Glu Val Cys Arg Arg His Glu Leu Tyr | | | |
| 290 | 295 | 300 | |
| Val Ser Phe Arg Asp Leu Gly Trp Leu Asp Trp Val Ile Ala Pro Gln | | | |
| 305 | 310 | 315 | 320 |
| Gly Tyr Ser Ala Tyr Tyr Cys Glu Gly Glu Cys Ala Phe Pro Leu Asp | | | |
| 325 | 330 | 335 | |
| Ser Cys Met Asn Ala Thr Asn His Ala Ile Leu Gln Ser Leu Val His | | | |
| 340 | 345 | 350 | |
| Leu Met Lys Pro Asp Val Val Pro Lys Ala Cys Cys Ala Pro Thr Lys | | | |
| 355 | 360 | 365 | |
| Leu Ser Ala Thr Ser Val Leu Tyr Tyr Asp Ser Ser Asn Asn Val Ile | | | |
| 370 | 375 | 380 | |
| Leu Arg Lys His Arg Asn Met Val Val Lys Ala Cys Gly Cys His | | | |
| 385 | 390 | 395 | |

(2) INFORMATION FOR SEQ ID NO:23:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1368 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 1..1368
- (D) OTHER INFORMATION: /label= "60A"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

| | | |
|---|--|-----|
| ATG TCG GGA CTG CGA AAC ACC TCG GAG GCC GTT GCA GTG CTC GCC TCC | | 48 |
| Met Ser Gly Leu Arg Asn Thr Ser Glu Ala Val Ala Val Leu Ala Ser | | |
| 1 5 10 15 | | |
| CTG GGA CTC GGA ATG GTT CTG CTC ATG TTC GTG GCG ACC ACG CCG CCG | | 96 |
| Leu Gly Leu Gly Met Val Leu Leu Met Phe Val Ala Thr Thr Pro Pro | | |
| 20 25 30 | | |
| GCC GTT GAG GCC ACC CAG TCG GGG ATT TAC ATA GAC AAC GGC AAG GAC | | 144 |
| Ala Val Glu Ala Thr Gln Ser Gly Ile Tyr Ile Asp Asn Gly Lys Asp | | |
| 35 40 45 | | |
| CAG ACG ATC ATG CAC AGA GTG CTG AGC GAG GAC GAC AAG CTG GAC GTC | | 192 |
| Gln Thr Ile Met His Arg Val Leu Ser Glu Asp Asp Lys Leu Asp Val | | |

- 70 -

| 50 | 55 | 60 | |
|--|-----|-----|-----|
| TCG TAC GAG ATC CTC GAG TTC CTG GGC ATC GCC GAA CGG CCG ACG CAC Ser Tyr Glu Ile Leu Glu Phe Leu Gly Ile Ala Glu Arg Pro Thr His | 65 | 70 | 240 |
| CTG AGC AGC CAC CAG TTG TCG CTG AGG AAG TCG GCT CCC AAG TTC CTG Leu Ser Ser His Gln Leu Ser Leu Arg Lys Ser Ala Pro Lys Phe Leu | 85 | 90 | 288 |
| CTG GAC GTC TAC CAC CGC ATC ACG GCG GAG GAG GGT CTC AGC GAT CAG Leu Asp Val Tyr His Arg Ile Thr Ala Glu Glu Gly Leu Ser Asp Gln | 100 | 105 | 336 |
| GAT GAG GAC GAC GAC TAC GAA CGC GGC CAT CGG TCC AGG AGG AGC GCC Asp Glu Asp Asp Asp Tyr Glu Arg Gly His Arg Ser Arg Arg Ser Ala | 115 | 120 | 384 |
| GAC CTC GAG GAG GAT GAG GGC GAG CAG CAG AAG AAC TTC ATC ACC GAC Asp Leu Glu Glu Asp Glu Gly Glu Gln Gln Lys Asn Phe Ile Thr Asp | 130 | 135 | 432 |
| CTG GAC AAG CGG GCC ATC GAC GAG AGC GAC ATC ATC ATG ACC TTC CTG Leu Asp Lys Arg Ala Ile Asp Glu Ser Asp Ile Ile Met Thr Phe Leu | 145 | 150 | 480 |
| AAC AAG CGC CAC CAC AAT GTG GAC GAA CTG CGT CAC GAG CAC GGC CGT Asn Lys Arg His His Asn Val Asp Glu Leu Arg His Glu His Gly Arg | 165 | 170 | 528 |
| CGC CTG TGG TTC GAC GTC TCC AAC GTG CCC AAC GAC AAC TAC CTG GTG Arg Leu Trp Phe Asp Val Ser Asn Val Pro Asn Asp Asn Tyr Leu Val | 180 | 185 | 576 |
| ATG GCC GAG CTG CGC ATC TAT CAG AAC GCC AAC GAG GGC AAG TGG CTG Met Ala Glu Leu Arg Ile Tyr Gln Asn Ala Asn Glu Gly Lys Trp Leu | 195 | 200 | 624 |
| ACC GCC AAC AGG GAG TTC ACC ATC ACG GTA TAC GCC ATT GGC ACC GGC Thr Ala Asn Arg Glu Phe Thr Ile Thr Val Tyr Ala Ile Gly Thr Gly | 210 | 215 | 672 |
| ACG CTG GGC CAG CAC ACC ATG GAG CCG CTG TCC TCG GTG AAC ACC ACC Thr Leu Gly Gln His Thr Met Glu Pro Leu Ser Ser Val Asn Thr Thr | 225 | 230 | 720 |
| GGG GAC TAC GTG GGC TGG TTG GAG CTC AAC GTG ACC GAG GGC CTG CAC Gly Asp Tyr Val Gly Trp Leu Glu Leu Asn Val Thr Glu Gly Leu His | 245 | 250 | 768 |
| GAG TGG CTG GTC AAG TCG AAG GAC AAT CAT GGC ATC TAC ATT GGA GCA Glu Trp Leu Val Lys Ser Lys Asp Asn His Gly Ile Tyr Ile Gly Ala | 260 | 265 | 816 |
| CAC GCT GTC AAC CGA CCC GAC CGC GAG GTG AAG CTG GAC GAC ATT GGA His Ala Val Asn Arg Pro Asp Arg Glu Val Lys Leu Asp Asp Ile Gly | 275 | 280 | 864 |
| | | 285 | |

- 71 -

| | |
|---|------|
| CTG ATC CAC CGC AAG GTG GAC GAC GAG TTC CAG CCC TTC ATG ATC GGC | 912 |
| Leu Ile His Arg Lys Val Asp Asp Glu Phe Gln Pro Phe Met Ile Gly | |
| 290 295 300 | |
| TTC TTC CGC GGA CCG GAG CTG ATC AAG GCG ACG GCC CAC AGC AGC CAC | 960 |
| Phe Phe Arg Gly Pro Glu Leu Ile Lys Ala Thr Ala His Ser Ser His | |
| 305 310 315 320 | |
| CAC AGG AGC AAG CGA AGC GCC AGC CAT CCA CGC AAG CGC AAG AAG TCG | 1008 |
| His Arg Ser Lys Arg Ser Ala Ser His Pro Arg Lys Arg Lys Lys Ser | |
| 325 330 335 | |
| GTG TCG CCC AAC AAC GTG CCG CTG CTG GAA CCG ATG GAG AGC ACG CCG | 1056 |
| Val Ser Pro Asn Asn Val Pro Leu Leu Glu Pro Met Glu Ser Thr Arg | |
| 340 345 350 | |
| AGC TGC CAG ATG CAG ACC CTG TAC ATA GAC TTC AAG GAT CTG GGC TGG | 1104 |
| Ser Cys Gln Met Gln Thr Leu Tyr Ile Asp Phe Lys Asp Leu Gly Trp | |
| 355 360 365 | |
| CAT GAC TGG ATC ATC GCA CCA GAG GGC TAT GGC GCC TTC TAC TGC AGC | 1152 |
| His Asp Trp Ile Ile Ala Pro Glu Gly Tyr Gly Ala Phe Tyr Cys Ser | |
| 370 375 380 | |
| GGC GAG TGC AAT TTC CCG CTC AAT GCG CAC ATG AAC GCC ACG AAC CAT | 1200 |
| Gly Glu Cys Asn Phe Pro Leu Asn Ala His Met Asn Ala Thr Asn His | |
| 385 390 395 400 | |
| GCG ATC GTC CAG ACC CTG GTC CAC CTG CTG GAG CCC AAG AAG GTG CCC | 1248 |
| Ala Ile Val Gln Thr Leu Val His Leu Leu Glu Pro Lys Lys Val Pro | |
| 405 410 415 | |
| AAG CCC TGC TGC GCT CCG ACC AGG CTG GGA GCA CTA CCC GTT CTG TAC | 1296 |
| Lys Pro Cys Cys Ala Pro Thr Arg Leu Gly Ala Leu Pro Val Leu Tyr | |
| 420 425 430 | |
| CAC CTG AAC GAC GAG AAT GTG AAC CTG AAA AAG TAT AGA AAC ATG ATT | 1344 |
| His Leu Asn Asp Glu Asn Val Asn Leu Lys Lys Tyr Arg Asn Met Ile | |
| 435 440 445 | |
| GTG AAA TCC TGC GGG TGC CAT TGA | 1368 |
| Val Lys Ser Cys Gly Cys His | |
| 450 455 | |

(2) INFORMATION FOR SEQ ID NO:24:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 455 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: prot in

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:

- 72 -

Met Ser Gly Leu Arg Asn Thr Ser Glu Ala Val Ala Val Leu Ala Ser
1 5 10 15

Leu Gly Leu Gly Met Val Leu Leu Met Phe Val Ala Thr Thr Pro Pro
20 25 30

Ala Val Glu Ala Thr Gln Ser Gly Ile Tyr Ile Asp Asn Gly Lys Asp
35 40 45

Gln Thr Ile Met His Arg Val Leu Ser Glu Asp Asp Lys Leu Asp Val
50 55 60

Ser Tyr Glu Ile Leu Glu Phe Leu Gly Ile Ala Glu Arg Pro Thr His
65 70 75 80

Leu Ser Ser His Gln Leu Ser Leu Arg Lys Ser Ala Pro Lys Phe Leu
85 90 95

Leu Asp Val Tyr His Arg Ile Thr Ala Glu Glu Gly Leu Ser Asp Gln
100 105 110

Asp Glu Asp Asp Asp Tyr Glu Arg Gly His Arg Ser Arg Arg Ser Ala
115 120 125

Asp Leu Glu Glu Asp Glu Gly Glu Gln Gln Lys Asn Phe Ile Thr Asp
130 135 140

Leu Asp Lys Arg Ala Ile Asp Glu Ser Asp Ile Ile Met Thr Phe Leu
145 150 155 160

Asn Lys Arg His His Asn Val Asp Glu Leu Arg His Glu His Gly Arg
165 170 175

Arg Leu Trp Phe Asp Val Ser Asn Val Pro Asn Asp Asn Tyr Leu Val
180 185 190

Met Ala Glu Leu Arg Ile Tyr Gln Asn Ala Asn Glu Gly Lys Trp Leu
195 200 205

Thr Ala Asn Arg Glu Phe Thr Ile Thr Val Tyr Ala Ile Gly Thr Gly
210 215 220

Thr Leu Gly Gln His Thr Met Glu Pro Leu Ser Ser Val Asn Thr Thr
225 230 235 240

Gly Asp Tyr Val Gly Trp Leu Glu Leu Asn Val Thr Glu Gly Leu His
245 250 255

Glu Trp Leu Val Lys Ser Lys Asp Asn His Gly Ile Tyr Ile Gly Ala
260 265 270

His Ala Val Asn Arg Pro Asp Arg Glu Val Lys Leu Asp Asp Ile Gly
275 280 285

Leu Ile His Arg Lys Val Asp Asp Glu Phe Gln Pro Phe Met Ile Gly
290 295 300

- 73 -

| | | | | | | | | | | | | | | | |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Phe | Phe | Arg | Gly | Pro | Glu | Leu | Ile | Lys | Ala | Thr | Ala | His | Ser | Ser | His |
| 305 | | | | 310 | | | | 315 | | | | 320 | | | |
| His | Arg | Ser | Lys | Arg | Ser | Ala | Ser | His | Pro | Arg | Lys | Arg | Lys | Ser | |
| | 325 | | | | 330 | | | 335 | | | | | | | |
| Val | Ser | Pro | Asn | Asn | Val | Pro | Leu | Leu | Glu | Pro | Met | Glu | Ser | Thr | Arg |
| | | 340 | | | | 345 | | | 350 | | | | | | |
| Ser | Cys | Gln | Met | Gln | Thr | Leu | Tyr | Ile | Asp | Phe | Lys | Asp | Leu | Gly | Trp |
| | | 355 | | | | 360 | | | 365 | | | | | | |
| His | Asp | Trp | Ile | Ile | Ala | Pro | Glu | Gly | Tyr | Gly | Ala | Phe | Tyr | Cys | Ser |
| | 370 | | | | 375 | | | 380 | | | | | | | |
| Gly | Glu | Cys | Asn | Phe | Pro | Leu | Asn | Ala | His | Met | Asn | Ala | Thr | Asn | His |
| | 385 | | | | 390 | | | 395 | | 400 | | | | | |
| Ala | Ile | Val | Gln | Thr | Leu | Val | His | Leu | Leu | Glu | Pro | Lys | Lys | Val | Pro |
| | | 405 | | | | 410 | | | 415 | | | | | | |
| Lys | Pro | Cys | Cys | Ala | Pro | Thr | Arg | Leu | Gly | Ala | Leu | Pro | Val | Leu | Tyr |
| | | 420 | | | | 425 | | | 430 | | | | | | |
| His | Leu | Asn | Asp | Glu | Asn | Val | Asn | Leu | Lys | Lys | Tyr | Arg | Asn | Met | Ile |
| | | 435 | | | 440 | | | 445 | | | | | | | |
| Val | Lys | Ser | Cys | Gly | Cys | His | | | | | | | | | |
| | | 450 | | | 455 | | | | | | | | | | |

(2) INFORMATION FOR SEQ ID NO:25:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1674 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 69..1268
- (D) OTHER INFORMATION: /note= "mOP3-pp"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:

| | |
|---|-----|
| GGATCCGCGG CGCTGTCCA TCCTTGTCTG CGAGGCCGTCG CTGGATGCGA GTCCGCTAAA | 60 |
| CGTCCGAG ATG GCT GCG CGT CCG GGA CTC CTA TGG CTA CTG GGC CTG GCT Met Ala Ala Arg Pro Gly Leu Leu Trp Leu Leu Gly Leu Ala | 110 |
| 1 5 10 | |
| CTG TGC GTG TTG GGC GGC GGT CAC CTC TCG CAT CCC CCG CAC GTC TTT | 158 |

- 74 -

| | | | | |
|---|-----|-----|-----|-----|
| Leu Cys Val Leu Gly Gly His Leu Ser His Pro Pro His Val Phe | | | | |
| 15 | 20 | 25 | 30 | |
| CCC CAG CGT CGA CTA GGA GTA CGC GAG CCC CGC GAC ATG CAG CGC GAG | | | | 206 |
| Pro Gln Arg Arg Leu Gly Val Arg Glu Pro Arg Asp Met Gln Arg Glu | | | | |
| 35 | 40 | 45 | | |
| ATT CGG GAG GTG CTG GGG CTA GCC GGG CGG CCC CGA TCC CGA GCA CGC | | | | 254 |
| Ile Arg Glu Val Leu Gly Leu Ala Gly Arg Pro Arg Ser Arg Ala Pro | | | | |
| 50 | 55 | 60 | | |
| GTC GGG GCT GCC CAG CAG CCA GCG TCT GCG CCC CTC TTT ATG TTG GAC | | | | 302 |
| Val Gly Ala Ala Gln Gln Pro Ala Ser Ala Pro Leu Phe Met Leu Asp | | | | |
| 65 | 70 | 75 | | |
| CTG TAC CGT GCC ATG ACG GAT GAC AGT GGC GGT GGG ACC CCG CAG CCT | | | | 350 |
| Leu Tyr Arg Ala Met Thr Asp Asp Ser Gly Gly Thr Pro Gln Pro | | | | |
| 80 | 85 | 90 | | |
| CAC TTG GAC CGT GCT GAC CTG ATT ATG AGC TTT GTC AAC ATA GTG GAA | | | | 398 |
| His Leu Asp Arg Ala Asp Leu Ile Met Ser Phe Val Asn Ile Val Glu | | | | |
| 95 | 100 | 105 | 110 | |
| CGC GAC CGT ACC CTG GGC TAC CAG GAG CCA CAC TGG AAG GAA TTC CAC | | | | 446 |
| Arg Asp Arg Thr Leu Gly Tyr Gln Glu Pro His Trp Lys Glu Phe His | | | | |
| 115 | 120 | 125 | | |
| TTT GAC CTA ACC CAG ATC CCT GCT GGG GAG GCT GTC ACA GCT GCT GAG | | | | 494 |
| Phe Asp Leu Thr Gln Ile Pro Ala Gly Glu Ala Val Thr Ala Ala Glu | | | | |
| 130 | 135 | 140 | | |
| TTC CGG ATC TAC AAA GAA CCC AGT ACC CAC CCG CTC AAC ACA ACC CTC | | | | 542 |
| Phe Arg Ile Tyr Lys Glu Pro Ser Thr His Pro Leu Asn Thr Thr Leu | | | | |
| 145 | 150 | 155 | | |
| CAC ATC AGC ATG TTC GAA GTG GTC CAA GAG CAC TCC AAC AGG GAG TCT | | | | 590 |
| His Ile Ser Met Phe Glu Val Val Gln Glu His Ser Asn Arg Glu Ser | | | | |
| 160 | 165 | 170 | | |
| GAC TTG TTC TTT TTG GAT CTT CAG ACG CTC CGA TCT GGG GAC GAG GGC | | | | 638 |
| Asp Leu Phe Phe Leu Asp Leu Gln Thr Leu Arg Ser Gly Asp Glu Gly | | | | |
| 175 | 180 | 185 | 190 | |
| TGG CTG GTG CTG GAC ATC ACA GCA GCC AGT GAC CGA TGG CTG CTG AAC | | | | 686 |
| Trp Leu Val Leu Asp Ile Thr Ala Ala Ser Asp Arg Trp Leu Leu Asn | | | | |
| 195 | 200 | 205 | | |
| CAT CAC AAG GAC CTA GGA CTC CGC CTC TAT GTG GAA ACC GAG GAT GGG | | | | 734 |
| His His Lys Asp Leu Gly Leu Arg Leu Tyr Val Glu Thr Glu Asp Gly | | | | |
| 210 | 215 | 220 | | |
| CAC AGC ATA GAT CCT GGC CTA GCT GGT CTG CTT GGA CGA CAA GCA CCA | | | | 782 |
| His Ser Ile Asp Pro Gly Leu Ala Gly Leu Leu Gly Arg Gln Ala Pro | | | | |
| 225 | 230 | 235 | | |
| CGC TCC AGA CAG CCT TTC ATG GTT GGT TTC AGG GCC AAC CAG AGT | | | | 830 |
| Arg Ser Arg Gln Pro Phe Met Val Gly Phe Phe Arg Ala Asn Gln Ser | | | | |

- 75 -

| 240 | 245 | 250 | |
|---|-----|-----|------|
| CCT GTG CGG GCC CCT CGA ACA GCA AGA CCA CTG AAG AAG AAG CAG CTA Pro Val Arg Ala Pro Arg Thr Ala Arg Pro Leu Lys Lys Lys Gln Leu 255 | 260 | 265 | 878 |
| AAT CAA ATC AAC CAG CTG CCG CAC TCC AAC AAA CAC CTA GGA ATC CTT Asn Gln Ile Asn Gln Leu Pro His Ser Asn Lys His Leu Gly Ile Leu 275 | 280 | 285 | 926 |
| GAT GAT GGC CAC GGT TCT CAC GGC AGA GAA GTT TGC CGC AGG CAT GAG Asp Asp Gly His Gly Ser His Gly Arg Glu Val Cys Arg Arg His Glu 290 | 295 | 300 | 974 |
| CTC TAT GTC AGC TTC CGT GAC CTT GGC TGG CTG GAC TCT GTC ATT GCC Leu Tyr Val Ser Phe Arg Asp Leu Gly Trp Leu Asp Ser Val Ile Ala 305 | 310 | 315 | 1022 |
| CCC CAG GGC TAC TCC GCC TAT TAC TGT GCT GGG GAG TGC ATC TAC CCA Pro Gln Gly Tyr Ser Ala Tyr Tyr Cys Ala Gly Glu Cys Ile Tyr Pro 320 | 325 | 330 | 1070 |
| CTG AAC TCC TGT ATG AAC TCC ACC AAC CAC GCC ACT ATG CAG GCC CTG Leu Asn Ser Cys Met Asn Ser Thr Asn His Ala Thr Met Gln Ala Leu 335 | 340 | 345 | 1118 |
| GTA CAT CTG ATG AAG CCA GAT ATC ATC CCC AAG GTG TGC TGT GTG CCT Val His Leu Met Lys Pro Asp Ile Ile Pro Lys Val Cys Cys Val Pro 355 | 360 | 365 | 1166 |
| ACT GAG CTG AGT GCC ATT TCT CTG CTC TAC TAT GAT AGA AAC AAT AAT Thr Glu Leu Ser Ala Ile Ser Leu Leu Tyr Tyr Asp Arg Asn Asn Asn 370 | 375 | 380 | 1214 |
| GTC ATC CTG CGC AGG GAG CGC AAC ATG GTA GTC CAG GCC TGT GGC TGC Val Ile Leu Arg Arg Glu Arg Asn Met Val Val Gln Ala Cys Gly Cys 385 | 390 | 395 | 1262 |
| CAC TGAGTCCCTG CCCAACAGCC TGCTGCCATC CCATCTATCT AGTCAGGCCT His 400 | | | 1315 |
| CTCTTCCAAG GCAGGAAACC AACAAAGAGG GAAGGCAGTG CTTTCAACTC CATGTCCACA | | | 1375 |
| TTCACAGTCT TGGCCCTCTC TGTTCTTTT GCCAAGGCTG AGAAGATGGT CCTAGTTATA | | | 1435 |
| ACCTGGTGA CCTCAGTAGC CCGATCTCTC ATCTCCCCAA ACTCCCCAAT GCAGCCAGGG | | | 1495 |
| GCATCTATGT CCTTTGGGAT TGGGCACAGA AGTCCAATT ACCAACTTAT TCATGAGTCA | | | 1555 |
| CTACTGGCCC AGCCTGGACT TGAACCTGGA ACACAGGGTA GAGCTCAGGC TCTTCAGTAT | | | 1615 |
| CCATCAGAAG ATTTAGGTGT GTGCAGACAT GACCACACTC CCCCTAGCAC TCCATAGCC | | | 1674 |

(2) INFORMATION FOR SEQ ID NO:26:

- 76 -

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 399 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:26:

Met Ala Ala Arg Pro Gly Leu Leu Trp Leu Leu Gly Leu Ala Leu Cys
1 5 10 15

Val Leu Gly Gly Gly His Leu Ser His Pro Pro His Val Phe Pro Gln
20 25 30

Arg Arg Leu Gly Val Arg Glu Pro Arg Asp Met Gln Arg Glu Ile Arg
35 40 45

Glu Val Leu Gly Leu Ala Gly Arg Pro Arg Ser Arg Ala Pro Val Gly
50 55 60

Ala Ala Gln Gln Pro Ala Ser Ala Pro Leu Phe Met Leu Asp Leu Tyr
65 70 75 80

Arg Ala Met Thr Asp Asp Ser Gly Gly Thr Pro Gln Pro His Leu
85 90 95

Asp Arg Ala Asp Leu Ile Met Ser Phe Val Asn Ile Val Glu Arg Asp
100 105 110

Arg Thr Leu Gly Tyr Gln Glu Pro His Trp Lys Glu Phe His Phe Asp
115 120 125

Leu Thr Gln Ile Pro Ala Gly Glu Ala Val Thr Ala Ala Glu Phe Arg
130 135 140

Ile Tyr Lys Glu Pro Ser Thr His Pro Leu Asn Thr Thr Leu His Ile
145 150 155 160

Ser Met Phe Glu Val Val Gln Glu His Ser Asn Arg Glu Ser Asp Leu
165 170 175

Phe Phe Leu Asp Leu Gln Thr Leu Arg Ser Gly Asp Glu Gly Trp Leu
180 185 190

Val Leu Asp Ile Thr Ala Ala Ser Asp Arg Trp Leu Leu Asn His His
195 200 205

Lys Asp Leu Gly Leu Arg Leu Tyr Val Glu Thr Glu Asp Gly His Ser
210 215 220

Ile Asp Pro Gly Leu Ala Gly Leu Leu Gly Arg Gln Ala Pro Arg Ser
225 230 235 240

Arg Gln Pro Ph Met Val Gly Phe Phe Arg Ala Asn Gln Ser Pro Val
245 250 255

- 77 -

| | | | | | | | | | | | | | | | |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Arg | Ala | Pro | Arg | Thr | Ala | Arg | Pro | Leu | Lys | Lys | Lys | Gln | Leu | Asn | Gln |
| | | | | | | | | 260 | 265 | | | | 270 | | |
| Ile | Asn | Gln | Leu | Pro | His | Ser | Asn | Lys | His | Gly | Ile | Leu | Asp | Asp | |
| | | | | | | | | 275 | 280 | | | | 285 | | |
| Gly | His | Gly | Ser | His | Gly | Arg | Glu | Val | Cys | Arg | Arg | His | Glu | Leu | Tyr |
| | | | | | | | | 290 | 295 | | | | 300 | | |
| Val | Ser | Phe | Arg | Asp | Leu | Gly | Trp | Leu | Asp | Ser | Val | Ile | Ala | Pro | Gln |
| | | | | | | | | 305 | 310 | | | | 315 | | 320 |
| Gly | Tyr | Ser | Ala | Tyr | Tyr | Cys | Ala | Gly | Glu | Cys | Ile | Tyr | Pro | Leu | Asn |
| | | | | | | | | 325 | 330 | | | | 335 | | |
| Ser | Cys | Met | Asn | Ser | Thr | Asn | His | Ala | Thr | Met | Gln | Ala | Leu | Val | His |
| | | | | | | | | 340 | 345 | | | | 350 | | |
| Leu | Met | Lys | Pro | Asp | Ile | Ile | Pro | Lys | Val | Cys | Cys | Val | Pro | Thr | Glu |
| | | | | | | | | 355 | 360 | | | | 365 | | |
| Leu | Ser | Ala | Ile | Ser | Leu | Leu | Tyr | Tyr | Asp | Arg | Asn | Asn | Asn | Val | Ile |
| | | | | | | | | 370 | 375 | | | | 380 | | |
| Leu | Arg | Arg | Glu | Arg | Asn | Met | Val | Val | Gln | Ala | Cys | Gly | Cys | His | |
| | | | | | | | | 385 | 390 | | | | 395 | | |

(2) INFORMATION FOR SEQ ID NO:27:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 104 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(ix) FEATURE:

- (A) NAME/KEY: Protein
- (B) LOCATION: 1..104
- (D) OTHER INFORMATION: /note= "BMP3"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:27:

| | | | | | | | | | | | | | | | |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Cys | Ala | Arg | Arg | Tyr | Leu | Lys | Val | Asp | Phe | Ala | Asp | Ile | Gly | Trp | Ser |
| 1 | | | | | 5 | | | | | | | 10 | | 15 | |

| | | | | | | | | | | | | | | | |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Glu | Trp | Ile | Ile | Ser | Pro | Lys | Ser | Phe | Asp | Ala | Tyr | Tyr | Cys | Ser | Gly |
| | | | | | | | | | | | | | | | 20 |
| | | | | | | | | | | | | | | | 25 |
| | | | | | | | | | | | | | | | 30 |

| | | | | | | | | | | | | | | | |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Ala | Cys | Gln | Phe | Pro | Met | Pro | Lys | Ser | Leu | Lys | Pro | Ser | Asn | His | Ala |
| | | | | | | | | | | | | | | | 35 |
| | | | | | | | | | | | | | | | 40 |
| | | | | | | | | | | | | | | | 45 |

| | | | | | | | | | | | | | | | |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Thr | Ile | Gln | Ser | Ile | Val | Ala | Arg | Ala | Val | Gly | Val | Val | Pro | Gly | Ile |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|

- 78 -

| | | |
|----|----|----|
| 50 | 55 | 60 |
|----|----|----|

| | | |
|---|-----|----|
| Pro Glu Pro Cys Cys Val Pro Glu Lys Met Ser Ser Leu Ser Ile Leu | 70 | 75 |
| 65 | | 80 |
| Phe Phe Asp Glu Asn Lys Asn Val Val Leu Lys Val Tyr Pro Asn Met | 85 | 90 |
| | | 95 |
| Thr Val Glu Ser Cys Ala Cys Arg | | |
| | 100 | |

(2) INFORMATION FOR SEQ ID NO:28:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 102 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: HOMO SAPIENS

(ix) FEATURE:

- (A) NAME/KEY: Protein
- (B) LOCATION: 1..102
- (D) OTHER INFORMATION: /note= "BMP5"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:28:

| | | |
|---|-----|----|
| Cys Lys Lys His Glu Leu Tyr Val Ser Phe Arg Asp Leu Gly Trp Gln | 10 | 15 |
| 1 | 5 | |
| Asp Trp Ile Ile Ala Pro Glu Gly Tyr Ala Ala Phe Tyr Cys Asp Gly | 25 | 30 |
| | | |
| Glu Cys Ser Phe Pro Leu Asn Ala His Met Asn Ala Thr Asn His Ala | 35 | 45 |
| | | |
| Ile Val Gln Thr Leu Val His Leu Met Phe Pro Asp His Val Pro Lys | 50 | 60 |
| | | |
| Pro Cys Cys Ala Pro Thr Lys Leu Asn Ala Ile Ser Val Leu Tyr Phe | 70 | 80 |
| 65 | | |
| Asp Asp Ser Ser Asn Val Ile Leu Lys Lys Tyr Arg Asn Met Val Val | 85 | 95 |
| | | |
| Arg Ser Cys Gly Cys His | | |
| | 100 | |

(2) INFORMATION FOR SEQ ID NO:29:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 102 amino acids

- 79 -

- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(vi) ORIGINAL SOURCE:
(A) ORGANISM: HOMO SAPIENS

(ix) FEATURE:
(A) NAME/KEY: Protein
(B) LOCATION: 1..102
(D) OTHER INFORMATION: /note= "BMP6"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:29:

Cys Arg Lys His Glu Leu Tyr Val Ser Phe Gln Asp Leu Gly Trp Gln
1 5 10 15

Asp Trp Ile Ile Ala Pro Lys Gly Tyr Ala Ala Asn Tyr Cys Asp Gly
20 25 30

Glu Cys Ser Phe Pro Leu Asn Ala His Met Asn Ala Thr Asn His Ala
35 40 45

Ile Val Gln Thr Leu Val His Leu Met Asn Pro Glu Tyr Val Pro Lys
50 55 60

Pro Cys Cys Ala Pro Thr Lys Leu Asn Ala Ile Ser Val Leu Tyr Phe
65 70 75 80

Asp Asp Asn Ser Asn Val Ile Leu Lys Lys Tyr Arg Trp Met Val Val
85 90 95

Arg Ala Cys Gly Cys His
100

(2) INFORMATION FOR SEQ ID NO:30:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 1247 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(vi) ORIGINAL SOURCE:
(A) ORGANISM: HOMO SAPIENS
(F) TISSUE TYPE: BRAIN

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 84..1199
(D) OTHER INFORMATION: /product= "GDF-1"
/note= "GDF-1 cDNA"

- 80 -

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:30:

| | |
|--|-----|
| GGGGACACCG GCCCCGCCCT CAGCCCCTG GTCCCGGGCC GCCGC GGACC CTGCGCACTC | 60 |
| TCTGGTCATC GCCTGGGAGG AAG ATG CCA CCG CCG CAG CAA GGT CCC TGC Met Pro Pro Pro Gln Gln Gly Pro Cys | 110 |
| 1 5 | |
| GGC CAC CAC CTC CTC CTC CTG GCC CTG CTG CTG CCC TCG CTG CCC Gly His His Leu Leu Leu Leu Ala Leu Leu Pro Ser Leu Pro | 158 |
| 10 15 20 25 | |
| CTG ACC CGC GCC CCC GTG CCC CCA GGC CCA GCC GCC CTG CTC CAG Leu Thr Arg Ala Pro Val Pro Pro Gly Pro Ala Ala Ala Leu Leu Gln | 206 |
| 30 35 40 | |
| GCT CTA GGA CTG CGC GAT GAG CCC CAG GGT GCC CCC AGG CTC CGG CCG Ala Leu Gly Leu Arg Asp Glu Pro Gln Gly Ala Pro Arg Leu Arg Pro | 254 |
| 45 50 55 | |
| GTT CCC CCG GTC ATG TGG CGC CTG TTT CGA CGC CGG GAC CCC CAG GAG Val Pro Pro Val Met Trp Arg Leu Phe Arg Arg Arg Asp Pro Gln Glu | 302 |
| 60 65 70 | |
| ACC AGG TCT GGC TCG CGG CGG ACG TCC CCA GGG GTC ACC CTG CAA CCG Thr Arg Ser Gly Ser Arg Arg Thr Ser Pro Gly Val Thr Leu Gln Pro | 350 |
| 75 80 85 | |
| TGC CAC GTG GAG GAG CTG GGG GTC GCC GGA AAC ATC GTG CGC CAC ATC Cys His Val Glu Leu Gly Val Ala Gly Asn Ile Val Arg His Ile | 398 |
| 90 95 100 105 | |
| CCG GAC CGC GGT GCG CCC ACC CGG GCC TCG GAG CCT GTC TCG GCC GCG Pro Asp Arg Gly Ala Pro Thr Arg Ala Ser Glu Pro Val Ser Ala Ala | 446 |
| 110 115 120 | |
| GGG CAT TGC CCT GAG TGG ACA GTC GTC TTC GAC CTG TCG GCT GTG GAA Gly His Cys Pro Glu Trp Thr Val Val Phe Asp Leu Ser Ala Val Glu | 494 |
| 125 130 135 | |
| CCC GCT GAG CGC CCG AGC CGG GCC CGC CTG GAG CTG CGT TTC GCG GCG Pro Ala Glu Arg Pro Ser Arg Ala Arg Leu Glu Leu Arg Phe Ala Ala | 542 |
| 140 145 150 | |
| GCG GCG GCG GCA GCC CCG GAG GGC GGC TGG GAG CTG AGC GTG GCG CAA Ala Ala Ala Ala Pro Glu Gly Gly Trp Glu Leu Ser Val Ala Gln | 590 |
| 155 160 165 | |
| GCG GGC CAG GGC GCG GGC GCG GAC CCC GGG CCG GTG CTG CTC CGC CAG Ala Gly Gln Gly Ala Gly Ala Asp Pro Gly Pro Val Leu Leu Arg Gln | 638 |
| 170 175 180 185 | |
| TTG GTG CCC GCC CTG GGG CCG CCA GTG CGC GCG GAG CTG CTG GGC GCC Leu Val Pro Ala Leu Gly Pro Pro Val Arg Ala Glu Leu Leu Gly Ala | 686 |
| 190 195 200 | |

- 81 -

| | | | | |
|--|-----|-----|-----|------|
| GCT TGG GCT CGC AAC GCC TCA TGG CCG CGC AGC CTC CGC CTG GCG CTG Ala Trp Ala Arg Asn Ala Ser Trp Pro Arg Ser Leu Arg Leu Ala Leu | 205 | 210 | 215 | 734 |
| GCG CTA CGC CCC CGG GCC CCT GCC GCC TGC GCG CGC CTG GCC GAG GCC Ala Leu Arg Pro Arg Ala Pro Ala Ala Cys Ala Arg Leu Ala Glu Ala | 220 | 225 | 230 | 782 |
| TCG CTG CTG CTG GTG ACC CTC GAC CCG CGC CTG TGC CAC CCC CTG GCC Ser Leu Leu Leu Val Thr Leu Asp Pro Arg Leu Cys His Pro Leu Ala | 235 | 240 | 245 | 830 |
| CGG CCG CGG CGC GAC GCC GAA CCC GTG TTG GGC GGC GGC CCC GGG GGC Arg Pro Arg Arg Asp Ala Glu Pro Val Leu Gly Gly Gly Pro Gly Gly | 250 | 255 | 260 | 878 |
| GCT TGT CGC GCG CGG CGG CTG TAC GTG AGC TTC CGC GAG GTG GGC TGG Ala Cys Arg Ala Arg Arg Leu Tyr Val Ser Phe Arg Glu Val Gly Trp | 270 | 275 | 280 | 926 |
| CAC CGC TGG GTC ATC GCG CCG CGC GGC TTC CTG GCC AAC TAC TGC CAG His Arg Trp Val Ile Ala Pro Arg Gly Phe Leu Ala Asn Tyr Cys Gln | 285 | 290 | 295 | 974 |
| GGT CAG TGC GCG CTG CCC GTC GCG CTG TCG GGG TCC GGG GGG CCG CCG Gly Gln Cys Ala Leu Pro Val Ala Leu Ser Gly Ser Gly Gly Pro Pro | 300 | 305 | 310 | 1022 |
| GCG CTC AAC CAC GCT GTG CTG CGC GCG CTC ATG CAC GCG GCC GCC CCG Ala Leu Asn His Ala Val Leu Arg Ala Leu Met His Ala Ala Ala Pro | 315 | 320 | 325 | 1070 |
| GGA GCC GCC GAC CTG CCC TGC TGC GTG CCC GCG CGC CTG TCG CCC ATC Gly Ala Ala Asp Leu Pro Cys Cys Val Pro Ala Arg Leu Ser Pro Ile | 330 | 335 | 340 | 1118 |
| TCC GTG CTC TTC TTT GAC AAC AGC GAC AAC GTG GTG CTG CGG CAG TAT Ser Val Leu Phe Phe Asp Asn Ser Asp Asn Val Val Leu Arg Gln Tyr | 350 | 355 | 360 | 1166 |
| GAG GAC ATG GTG GTG GAC GAG TGC GGC TGC CGC TAACCCGGGG CGGGCAGGGAA Glu Asp Met Val Val Asp Glu Cys Gly Cys Arg | 365 | 370 | | 1219 |
| CCCCGGGCCA ACAATAAATG CGCGCGTGG | | | | 1247 |

(2) INFORMATION FOR SEQ ID NO:31:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 372 amino acids
 - (B) TYPE: amino acid
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

- 82 -

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:31:

Met Pro Pro Pro Gln Gln Gly Pro Cys Gly His His Leu Leu Leu
1 5 10 15

Leu Ala Leu Leu Leu Pro Ser Leu Pro Leu Thr Arg Ala Pro Val Pro
20 25 30

Pro Gly Pro Ala Ala Ala Leu Leu Gln Ala Leu Gly Leu Arg Asp Glu
35 40 45

Pro Gln Gly Ala Pro Arg Leu Arg Pro Val Pro Pro Val Met Trp Arg
50 55 60

Leu Phe Arg Arg Arg Asp Pro Gln Glu Thr Arg Ser Gly Ser Arg Arg
65 70 75 80

Thr Ser Pro Gly Val Thr Leu Gln Pro Cys His Val Glu Glu Leu Gly
85 90 95

Val Ala Gly Asn Ile Val Arg His Ile Pro Asp Arg Gly Ala Pro Thr
100 105 110

Arg Ala Ser Glu Pro Val Ser Ala Ala Gly His Cys Pro Glu Trp Thr
115 120 125

Val Val Phe Asp Leu Ser Ala Val Glu Pro Ala Glu Arg Pro Ser Arg
130 135 140

Ala Arg Leu Glu Leu Arg Phe Ala Ala Ala Ala Ala Ala Pro Glu
145 150 155 160

Gly Gly Trp Glu Leu Ser Val Ala Gln Ala Gly Gln Gly Ala Gly Ala
165 170 175

Asp Pro Gly Pro Val Leu Leu Arg Gln Leu Val Pro Ala Leu Gly Pro
180 185 190

Pro Val Arg Ala Glu Leu Leu Gly Ala Ala Trp Ala Arg Asn Ala Ser
195 200 205

Trp Pro Arg Ser Leu Arg Leu Ala Leu Ala Leu Arg Pro Arg Ala Pro
210 215 220

Ala Ala Cys Ala Arg Leu Ala Glu Ala Ser Leu Leu Leu Val Thr Leu
225 230 235 240

Asp Pro Arg Leu Cys His Pro Leu Ala Arg Pro Arg Arg Asp Ala Glu
245 250 255

Pro Val Leu Gly Gly Pro Gly Gly Ala Cys Arg Ala Arg Arg Leu
260 265 270

Tyr Val Ser Phe Arg Glu Val Gly Trp His Arg Trp Val Ile Ala Pro
275 280 285

Arg Gly Phe Leu Ala Asn Tyr Cys Gln Gly Gln Cys Ala Leu Pro Val

- 83 -

290

295

300

Ala Leu Ser Gly Ser Gly Gly Pro Pro Ala Leu Asn His Ala Val Leu
305 310 315 320

Arg Ala Leu Met His Ala Ala Ala Pro Gly Ala Ala Asp Leu Pro Cys
325 330 335

Cys Val Pro Ala Arg Leu Ser Pro Ile Ser Val Leu Phe Phe Asp Asn
340 345 350

Ser Asp Asn Val Val Leu Arg Gln Tyr Glu Asp Met Val Val Asp Glu
355 360 365

Cys Gly Cys Arg
370

CLAIMS

What is claimed is:

1. 1. A method of treatment for a mammal in, or at risk of, chronic renal failure comprising administering to said mammal a therapeutically effective amount of an OP/BMP renal therapeutic agent or morphogen.
1. 2. A method of treatment for a mammal in, or at risk of, chronic renal failure comprising administering to said mammal a therapeutically effective amount of an inducer of endogenous OP/BMP renal therapeutic agent or morphogen expression.
1. 3. A method of treatment for a mammal in, or at risk of, chronic renal failure comprising administering to said mammal a therapeutically effective amount of an agonist of an OP/BMP renal therapeutic agent or morphogen receptor.
1. 4. A method of treatment for a mammal in, or at risk of, chronic renal failure comprising introducing within the kidney of said mammal a therapeutically effective amount of renal mesenchymal progenitor cells.
1. 5. A method as in claim 4 comprising the additional step of inducing metanephric differentiation of said cells by contacting said cells with an OP/BMP renal therapeutic agent or morphogen.
1. 6. A method as in claim 4 comprising the additional step of inducing metanephric differentiation of said cells by contacting said cells with an inducer of an OP/BMP renal therapeutic agent or morphogen.
1. 7. A method as in claim 4 comprising the additional step of inducing metanephric differentiation of said cells by contacting said cells with an agonist of an OP/BMP renal therapeutic agent or morphogen receptor.
1. 8. A method of treatment to delay the need for, or reduce the frequency of, chronic dialysis treatments comprising administering to a mammal a therapeutically effective amount of an OP/BMP renal therapeutic agent or morphogen.
1. 9. A method of treatment to delay the need for, or reduce the frequency of, chronic dialysis treatments comprising

- 3 administering to said mammal a therapeutically effective amount of an inducer of
4 endogenous OP/BMP renal therapeutic agent or morphogen expression.
- 1 10. A method of treatment to delay the need for, or reduce the frequency of, chronic dialysis
2 treatments comprising
3 administering to said mammal a therapeutically effective amount of an agonist of an
4 OP/BMP renal therapeutic agent or morphogen receptor.
- 1 11. A method as in any one of claims 1-10 wherein
2 said mammal is afflicted with a condition selected from the group consisting of chronic
3 renal failure, end-stage renal disease, chronic diabetic nephropathy, diabetic glomerulopathy,
4 diabetic renal hypertrophy, hypertensive nephrosclerosis, hypertensive glomerulosclerosis, chronic
5 glomerulonephritis, hereditary nephritis, and renal dysplasia.
- 1 12. A method as in any one of claims 1-10 wherein
2 examination of a renal biopsy of said mammal indicates that said mammal is afflicted with
3 a condition selected from the group consisting of glomerular hypertrophy, tubular hypertrophy,
4 glomerulosclerosis, and tubulointerstitial sclerosis.
- 1 13. A method as in any one of claims 1-10 wherein
2 examination of said mammal indicates renal fibrosis.
- 1 14. A method as in claim 13 wherein
2 said examination is an ultrasound, MRI or CAT scan of said mammal.
- 1 15. A method as in any one of claims 1-10 wherein
2 said mammal possesses a number of functional nephron units which is less than about 50%
3 of a number of functional nephron units present in a mammal having intact healthy kidneys.
- 1 16. A method as in any one of claims 1-10 wherein
2 said mammal possesses a number of functional nephron units which is less than about 40%
3 of a number of functional nephron units present in a mammal having intact healthy kidneys.
- 1 17. A method as in any one of claims 1-10 wherein
2 said mammal possesses a number of functional nephron units which is less than about 30%
3 of a number of functional nephron units present in a mammal having intact healthy kidneys.
- 1 18. A method as in any one of claims 1-10 wherein

2 said mammal possesses a number of functional nephron units which is less than about 20%
3 of a number of functional nephron units present in a mammal having intact healthy kidneys.

1 19. A method as in any one of claims 1-10 wherein
2 said mammal is a kidney transplant recipient.

1 20. A method as in any one of claims 1-10 wherein
2 said mammal possesses only one kidney.

1 21. A method as in any one of claims 1-10 wherein
2 examination of a urinary sediment of said mammal indicates a presence of broad casts.

1 22. A method as in any one of claims 1-10 wherein
2 said mammal has a GFR which is chronically less than about 50% of a GFR_{exp} for said
3 mammal.

1 23. A method as in claim 22 wherein
2 said mammal has a GFR which is chronically less than about 40% of a GFR_{exp} for said
3 mammal.

1 24. A method as in claim 22 wherein
2 said mammal has a GFR which is chronically less than about 30% of a GFR_{exp} for said
3 mammal.

1 25. A method as in claim 22 wherein
2 said mammal has a GFR which is chronically less than about 20% of a GFR_{exp} for said
3 mammal.

1 26. A method as in any one of claims 1-10 wherein
2 said mammal is a human male weighing at least about 50 kg and has a GFR which is
3 chronically less than about 50 ml/min.

1 27. A method as in claim 26 wherein
2 said mammal is a human male weighing at least about 50 kg and has a GFR which is
3 chronically less than about 40 ml/min.

1 28. A method as in claim 26 wherein
2 said mammal is a human male weighing at least about 50 kg and has a GFR which is
3 chronically less than about 30 ml/min.

- 1 29. A method as in claim 26 wherein
2 said mammal is a human male weighing at least about 50 kg and has a GFR which is
3 chronically less than about 20 ml/min.
- 1 30. A method as in any one of claims 1-10 wherein
2 said mammal is a human female weighing at least about 40 kg and has a GFR which is
3 chronically less than about 40 ml/min.
- 1 31. A method as in claim 30 wherein
2 said mammal is a human female weighing at least about 40 kg and has a GFR which is
3 chronically less than about 30 ml/min.
- 1 32. A method as in claim 30 wherein
2 said mammal is a human female weighing at least about 40 kg and has a GFR which is
3 chronically less than about 20 ml/min.
- 1 33. A method as in claim 30 wherein
2 said mammal is a human female weighing at least about 40 kg and has a GFR which is
3 chronically less than about 10 ml/min.
- 1 34. A method as in any one of claims 1-10 wherein said treatment reduces serum creatinine
2 levels in said mammal by at least about 5% over 3 months.
- 1 35. A method as in any one of claims 1-10 wherein
2 prior to said treatment said mammal presented a chronic decline in a clinical indicator of
3 renal function; and
4 after at least about 3 months of said treatment, said indicator stabilizes.
- 1 36. A method as in any one of claims 1-3 wherein said administration is oral.
- 1 37. A method as in any one of claims 1-3 wherein said administration is parenteral.
- 1 38. A method as in claim 37 wherein said administration is intravenous.
- 1 39. A method as in claim 37 wherein said administration is intraperitoneal.
- 1 40. A method as in claim 37 wherein said administration is into the renal capsule.
- 1 41. A method as in claim 37 wherein a stent has been implanted into said mammal for said
2 administration.
- 1 42. A method as in claim 41 wherein said stent is an intravenous stent.

- 1 43. A method as in claim 41 wherein said stent is an intraperitoneal stent.
- 1 44. A method as in claim 41 wherein said stent is a renal intracapsular stent.
- 1 45. A method as in claim 37 wherein said administration is by an implanted device.
- 1 46. A method as in any one of claims 1-3 wherein said administration is at least once a week
2 for a period of at least about one month.
- 1 47. A method as in any one of claims 1-3 wherein said administration is at least once a month
2 for a period of at least about one year.
- 1 48. A method as in claim 1 wherein said OP/BMP renal therapeutic agent or morphogen is
2 administered at a dosage of about 0.01-1000 µg/kg body weight of said mammal.
- 1 49. A method as in claim 48 wherein said OP/BMP renal therapeutic agent or morphogen is
2 administered at a dosage of about 10-300 µg/kg body weight of said mammal.
- 1 50. A method of promoting metanephric differentiation of renal mesenchymal progenitor cells
2 comprising the step of contacting said cells with an OP/BMP renal therapeutic agent or
3 morphogen in an amount effective to induce said differentiation.
- 1 51. A method as in claim 1 wherein said renal therapeutic agent comprises a polypeptide
2 consisting of at least a C-terminal cysteine domain of a protein selected from the group consisting
3 of a pro form, a mature form, and a soluble form of a polypeptide selected from the group
4 consisting of OP-1, OP-2, OP-3, BMP2, BMP3, BMP4, BMP5, BMP6, and BMP9.
- 1 52. A method as in claim 51 wherein said renal therapeutic agent comprises a polypeptide
2 consisting of at least a C-terminal cysteine domain of a protein selected from the group consisting
3 of a pro form, a mature form, and a soluble form of human OP-1.
- 1 53. A method as in claim 1 wherein said renal therapeutic agent comprises a polypeptide
2 having at least 70% homology with an amino acid sequence of a C-terminal seven-cysteine
3 domain of human OP-1.
- 1 54. A method as in claim 53 wherein said polypeptide has at least 75% homology with an
2 amino acid sequence of a C-terminal seven-cysteine domain of human OP-1.
- 1 55. A method as in claim 53 wherein said polypeptide has at least 80% homology with an
2 amino acid sequence of a C-terminal seven-cysteine domain of human OP-1.
- 1 56. A method as in claim 53 wherein said polypeptide has at least 60% identity with an amino
2 acid sequence of a C-terminal seven-cysteine domain of human OP-1.

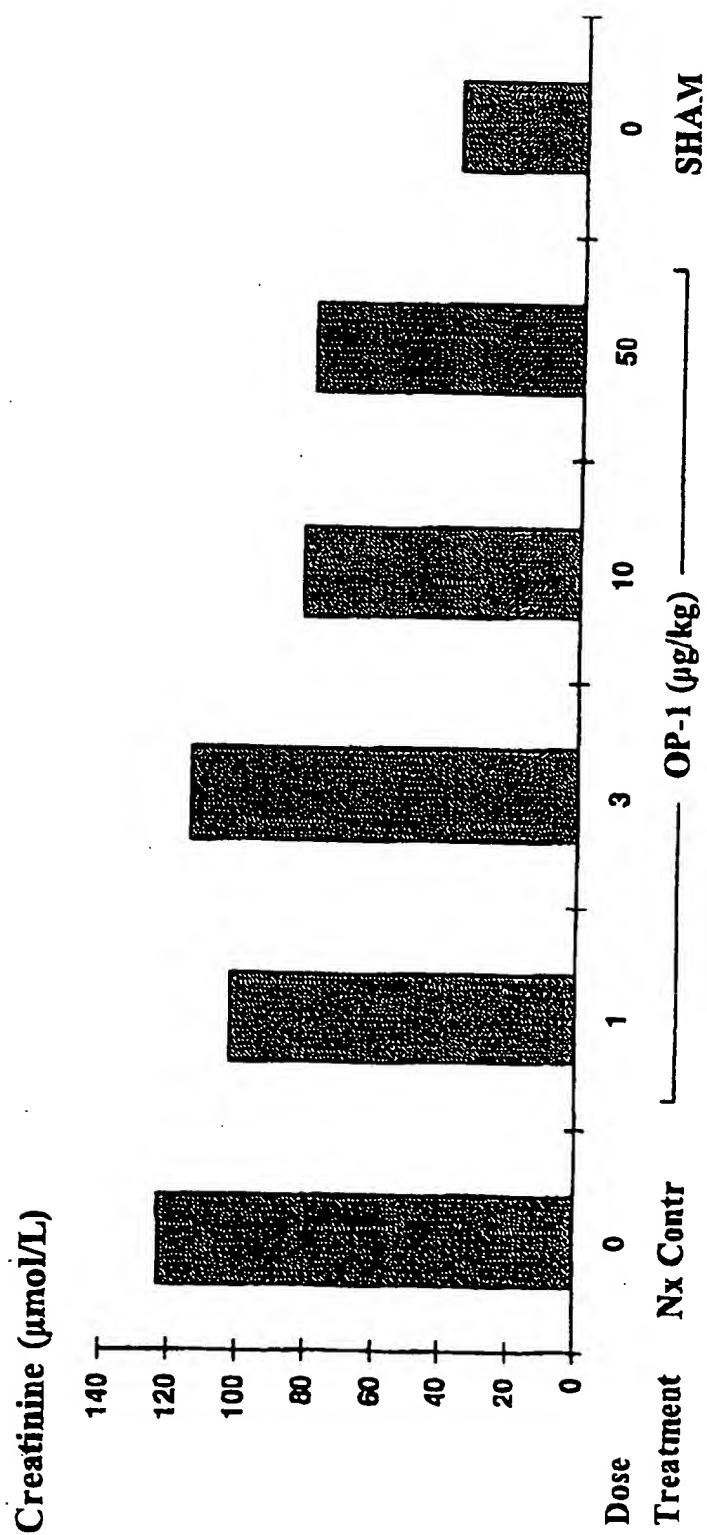


Figure 1

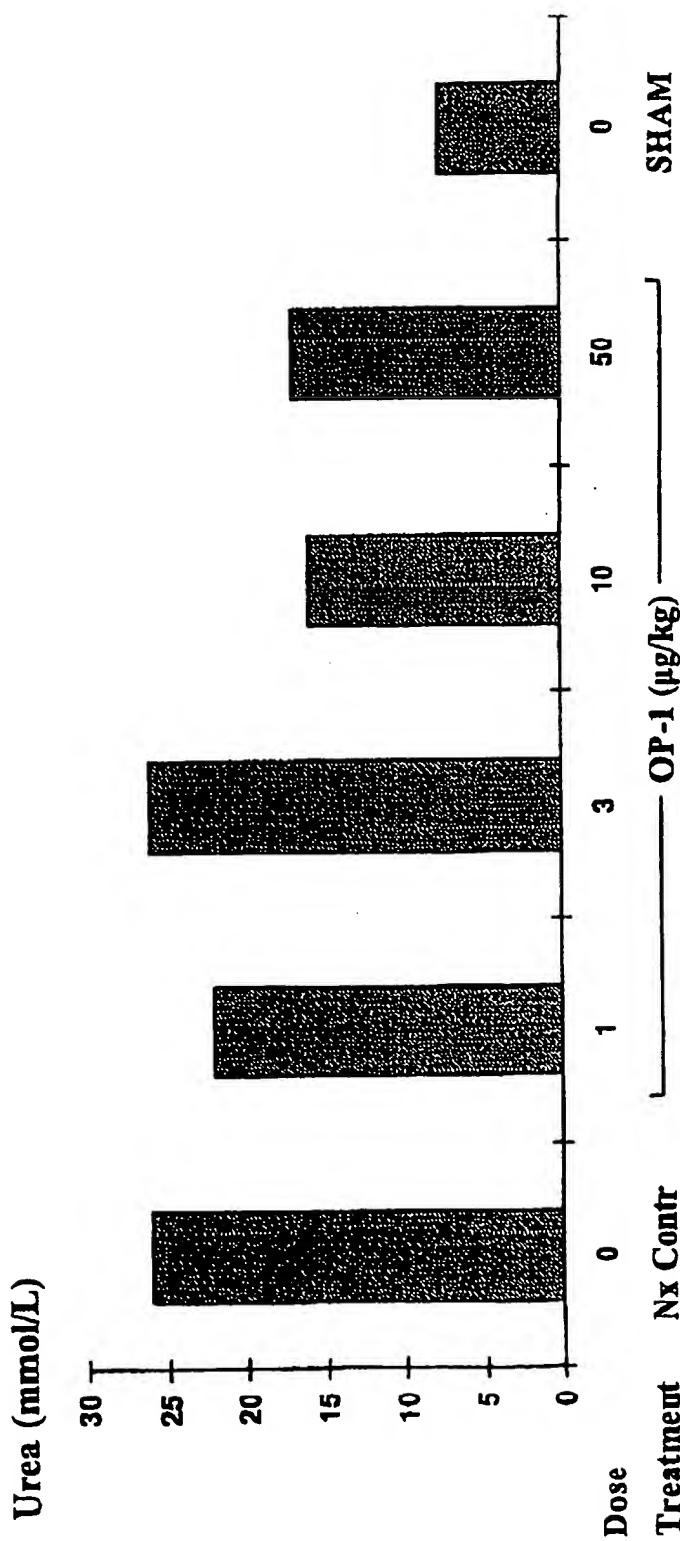
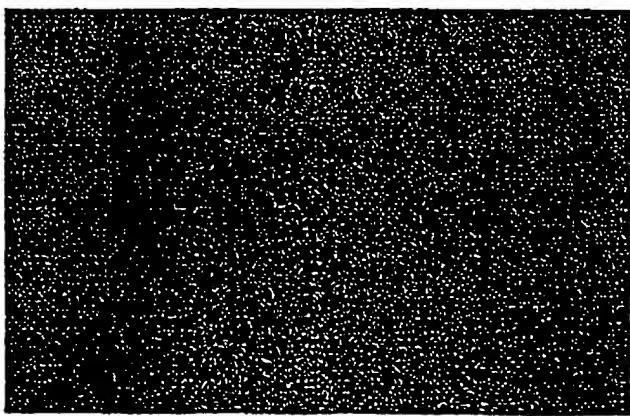


Figure 2

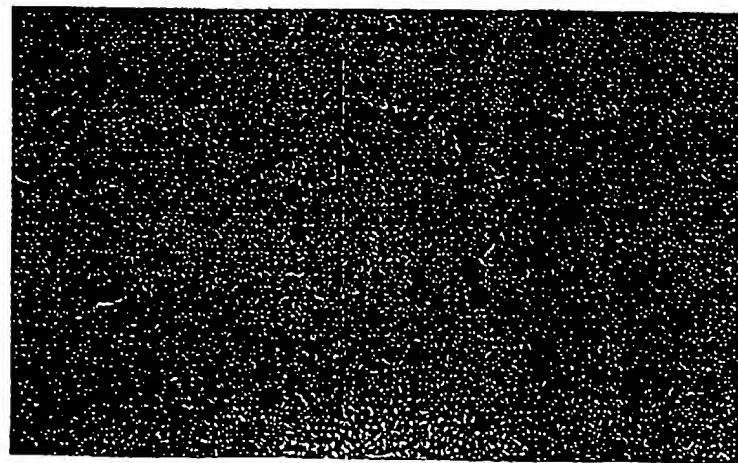


Figure 3

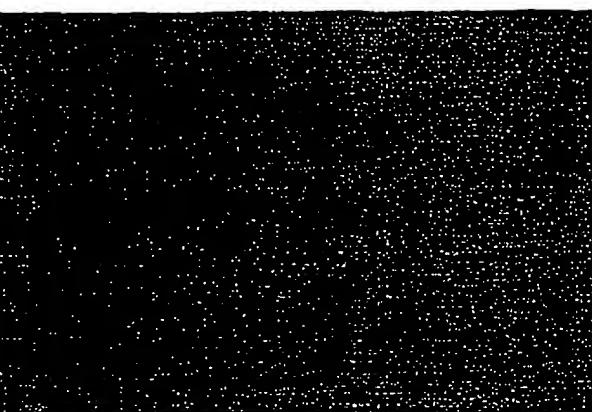
B



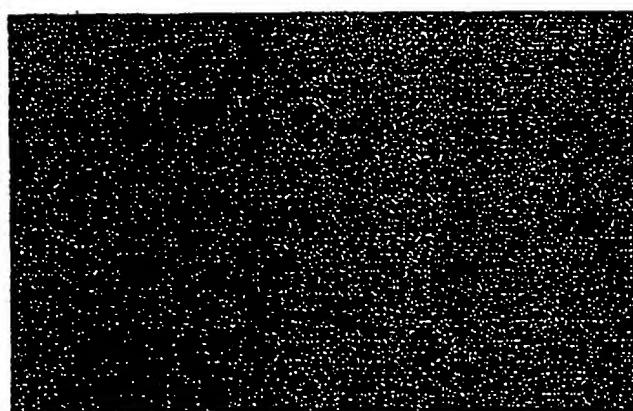
A



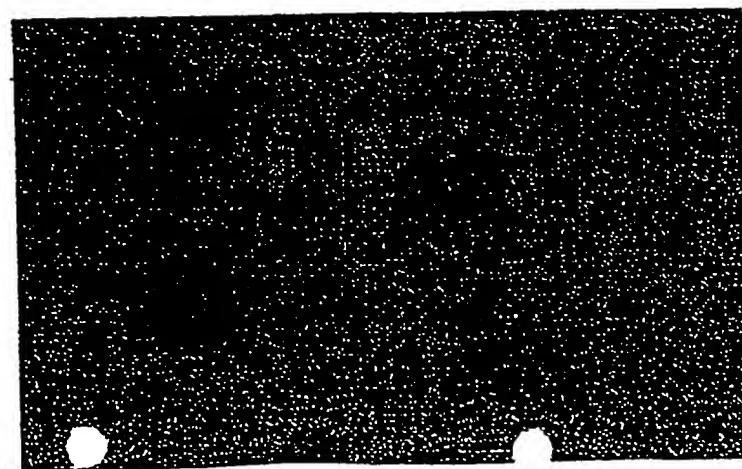
C



C



B



A

Figure 4

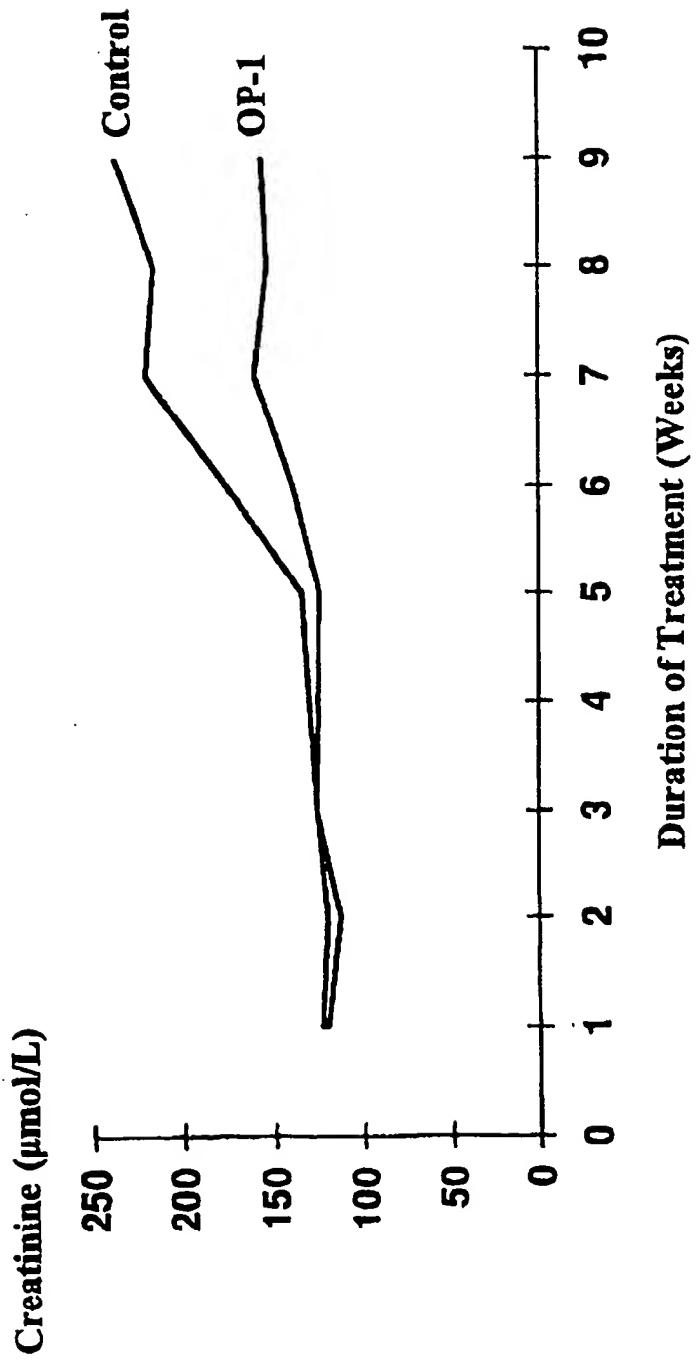


Figure 5

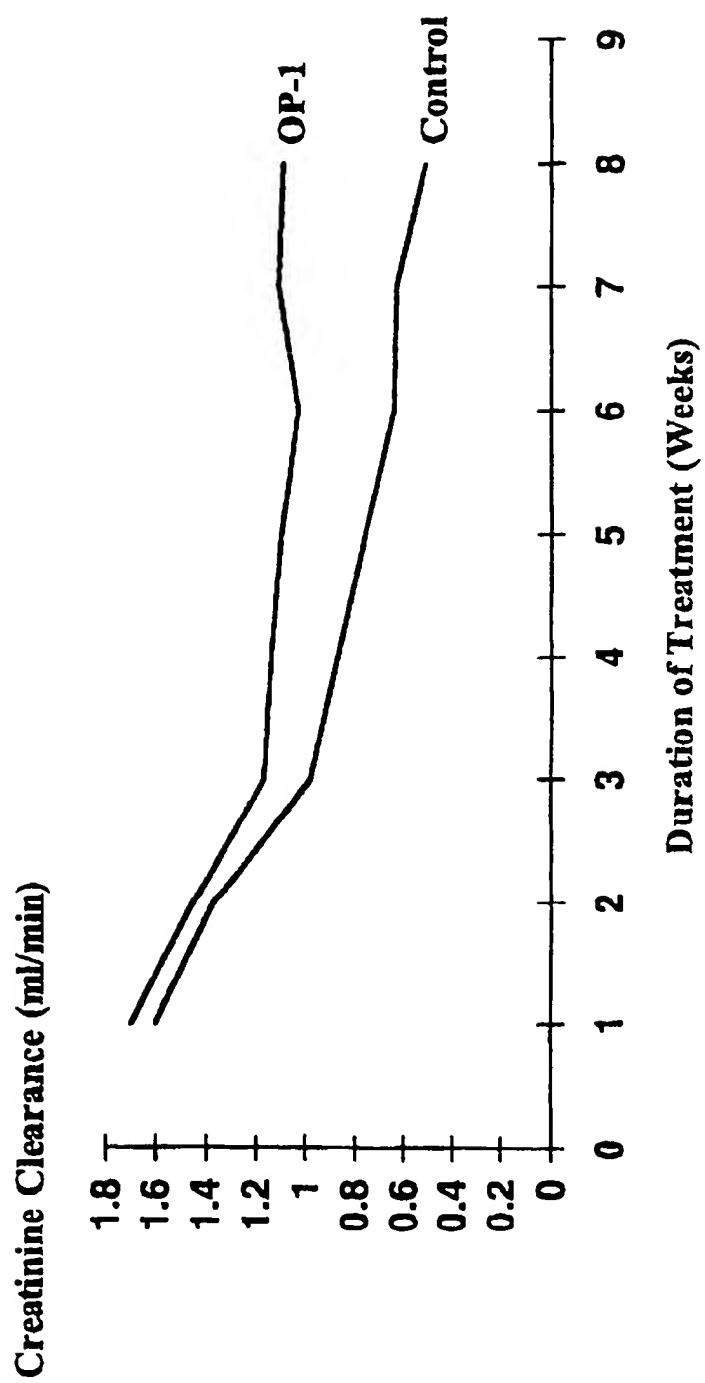


Figure 6

| | Cys | Lys | Lys | His | Glu | Leu | Tyr | Val |
|---------|-----|-----|-----|-----|-----|-----|-----|-----|
| hOP-1 | ... | ... | ... | ... | ... | ... | ... | ... |
| mOP-1 | ... | ... | Arg | Arg | ... | ... | ... | ... |
| hOP-2 | ... | ... | Arg | Arg | ... | ... | ... | ... |
| mOP-2 | ... | ... | Arg | Arg | ... | ... | ... | ... |
| mOP-3 | ... | ... | Arg | Arg | ... | ... | ... | ... |
| DPP | ... | ... | Arg | Arg | ... | ... | ... | ... |
| Vgl | ... | ... | ... | Lys | Arg | His | ... | ... |
| Vgr-1 | ... | ... | ... | ... | ... | Gly | ... | ... |
| CBMP-2A | ... | ... | Arg | ... | Pro | ... | ... | ... |
| CBMP-2B | ... | Arg | Arg | ... | Ser | ... | ... | ... |
| BMP3 | ... | Ala | Arg | Arg | Tyr | ... | Lys | ... |
| GDF-1 | ... | Arg | Ala | Arg | Arg | ... | ... | ... |
| 60A | ... | Gln | Met | Glu | Thr | ... | ... | ... |
| BMP5 | ... | ... | ... | ... | ... | ... | ... | ... |
| BMP6 | ... | Arg | ... | ... | ... | ... | ... | ... |

5

1

FIGURE 7-1

| | | | | | | | | | |
|---------|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| hOP-1 | Ser | Phe | Arg | Asp | Leu | Gly | Trp | Gln | Asp |
| mOP-1 | ... | ... | ... | ... | ... | ... | ... | ... | ... |
| hOP-2 | ... | ... | Gln | ... | ... | ... | ... | Leu | ... |
| mOP-2 | Ser | ... | ... | ... | ... | ... | ... | Leu | ... |
| mOP-3 | ... | ... | ... | ... | ... | ... | ... | Leu | ... |
| DPP | Asp | ... | Ser | ... | Val | ... | ... | Asp | ... |
| Vg1 | Glu | ... | Lys | ... | Val | ... | ... | Asn | ... |
| Vgr-1 | ... | ... | Gln | ... | Val | ... | ... | ... | ... |
| CBMP-2A | Asp | ... | Ser | ... | Val | ... | ... | Asn | ... |
| CBMP-2B | Asp | ... | Ser | ... | Val | ... | ... | Asn | ... |
| BMP3 | Asp | ... | Ala | ... | Ile | ... | ... | Ser | Glu |
| GDF-1 | ... | ... | ... | Glu | Val | ... | ... | His | Arg |
| 60A | Asp | ... | Lys | ... | ... | ... | ... | His | ... |
| BMP5 | ... | ... | ... | ... | ... | ... | ... | ... | ... |
| BMP6 | ... | ... | Gln | ... | ... | ... | ... | ... | ... |
| | | | | 10 | | | | | 15 |

FIGURE 7-2

| | | | | | | | | | |
|---------|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| hOP-1 | Trp | Ile | Ile | Ala | Pro | Glu | Gly | Tyr | Ala |
| mOP-1 | ... | ... | ... | ... | ... | ... | ... | ... | ... |
| hOP-2 | ... | Val | ... | ... | Gln | ... | ... | Ser | |
| mOP-2 | ... | Val | ... | ... | Gln | ... | ... | Ser | |
| mOP-3 | Ser | Val | ... | ... | Gln | ... | ... | Ser | |
| DPP | ... | ... | Val | ... | Leu | ... | ... | Asp | |
| Vg1 | ... | Val | ... | ... | Gln | ... | ... | Met | |
| Vgr-1 | ... | ... | ... | ... | Lys | ... | ... | ... | |
| CBMP-2A | ... | ... | Val | ... | Pro | ... | ... | His | |
| CBMP-2B | ... | ... | Val | ... | Pro | ... | ... | Gln | |
| BMP3 | ... | ... | Ser | ... | Lys | Ser | Phe | Asp | |
| GDF-1 | ... | Val | ... | ... | Arg | ... | Phe | Leu | |
| 60A | ... | ... | ... | ... | ... | ... | ... | Gly | |
| BMP5 | ... | ... | ... | ... | ... | ... | ... | ... | |
| BMP6 | ... | ... | ... | ... | ... | LYS | ... | ... | |
| | | | | | | | | | 25 |

FIGURE 7-3

| | | | | | | | | | |
|---------|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| hOP-1 | Ala | Tyr | Tyr | Cys | Glu | Gly | Glu | Cys | Ala |
| mOP-1 | ... | ... | ... | ... | ... | ... | ... | ... | ... |
| hOP-2 | ... | ... | ... | ... | ... | ... | ... | ... | Ser |
| mOP-2 | ... | ... | ... | ... | ... | ... | ... | ... | ... |
| mOP-3 | ... | ... | ... | ... | Ala | ... | ... | ... | Ile |
| DPP | ... | ... | ... | ... | His | ... | Lys | ... | Pro |
| Vgr1 | ... | Asn | ... | ... | Tyr | ... | ... | ... | Pro |
| Vgr-1 | ... | Asn | ... | Asp | ... | ... | ... | Ser | ... |
| CBMP-2A | ... | Phe | ... | His | ... | Glu | ... | Pro | ... |
| CBMP-2B | ... | Phe | ... | His | ... | Asp | ... | Pro | ... |
| BMP3 | ... | ... | ... | Ser | ... | Ala | ... | Gln | ... |
| GDF-1 | ... | Asn | ... | Gln | ... | Gln | ... | ... | ... |
| 60A | ... | Phe | ... | Ser | ... | ... | ... | Asn | ... |
| BMP5 | ... | Phe | ... | Asp | ... | ... | ... | Ser | ... |
| BMP6 | ... | Asn | ... | Asp | ... | ... | ... | Ser | ... |
| | | | | | 30 | | 35 | | |

FIGURE 7A

| | | | | | | | | | |
|---------|-----|-----|-----|-----|-----|-----|-----|-------|-----|
| hOP-1 | Phe | Pro | Leu | Asn | Ser | Tyr | Met | Asn | Ala |
| mOP-1 | ... | ... | ... | ... | ... | ... | ... | ... | ... |
| hOP-2 | ... | ... | ... | Asp | ... | Cys | ... | ... | ... |
| mOP-2 | ... | ... | ... | Asp | ... | Cys | ... | ... | ... |
| mOP-3 | Tyr | ... | ... | ... | ... | Cys | ... | ... | Ser |
| DPP | ... | ... | ... | Ala | Asp | His | Phe | ... | Ser |
| Vg1 | Tyr | ... | ... | Thr | Glu | Ile | Leu | ... | Gly |
| Vgr-1 | ... | ... | ... | Ala | His | ... | ... | ... | ... |
| CBMP-2A | ... | ... | ... | Ala | Asp | His | Leu | ... | Ser |
| CBMP-2B | ... | ... | ... | Ala | Asp | His | Leu | ... | Ser |
| GDF-1 | Leu | ... | Val | Ala | Leu | Ser | Gly | Ser** | ... |
| BMP3 | ... | ... | Met | Pro | Lys | Ser | Leu | Lys | Pro |
| 60A | ... | ... | ... | ... | Ala | His | ... | ... | ... |
| BMP5 | ... | ... | ... | ... | Ala | His | Met | ... | ... |
| BMP6 | ... | ... | ... | ... | Ala | His | Met | ... | ... |

| | | | | | | | | | |
|---------|-----|-----|-----|-----|-----|-----|-----|-----|---------|
| hOP-1 | Thr | Asn | His | Ala | Ile | Val | Gln | Thr | Leu |
| mOP-1 | ... | ... | ... | ... | ... | ... | ... | ... | ... |
| hOP-2 | ... | ... | ... | ... | ... | Leu | ... | Ser | ... |
| mOP-2 | ... | ... | ... | ... | ... | Leu | ... | Ser | ... |
| mOP-3 | ... | ... | ... | ... | ... | Thr | Met | ... | Ala |
| DPP | ... | ... | ... | ... | ... | Val | ... | ... | ... |
| Vgl | Ser | ... | ... | ... | ... | Leu | ... | ... | ... |
| Vgr-1 | ... | ... | ... | ... | ... | ... | ... | ... | ... |
| CBMP-2A | ... | ... | ... | ... | ... | ... | ... | ... | ... |
| CBMP-2B | ... | ... | ... | ... | ... | ... | ... | ... | ... |
| BMP3 | Ser | ... | ... | ... | ... | Thr | Ile | ... | Ser Ile |
| GDF-1 | Leu | ... | ... | ... | ... | Val | Leu | Arg | Ala |
| 60A | ... | ... | ... | ... | ... | ... | ... | ... | ... |
| BMP5 | ... | ... | ... | ... | ... | ... | ... | ... | ... |
| BMP6 | ... | ... | ... | ... | ... | ... | ... | ... | ... |
| | | | | | | | | 45 | 50 |

FIGURE 7-6

| | | | | | | | | | |
|---------|-----|-----|------|-----|-----|-----|-----|-----|-----|
| hOP-1 | Val | His | Phe | Ile | Asn | Pro | Glu | Thr | Val |
| mOP-1 | ... | ... | ... | ... | ... | ... | Asp | ... | ... |
| hOP-2 | ... | His | Leu | Met | Lys | ... | Asn | Ala | ... |
| mOP-2 | ... | His | Leu | Met | Lys | ... | Asp | Val | ... |
| mOP-3 | ... | ... | Leu | Met | Lys | ... | Asp | Ile | Ile |
| DPP | ... | Asn | Asn | Asn | ... | ... | Gly | Lys | ... |
| Vgr-1 | ... | ... | Ser | ... | Glu | ... | ... | Asp | Ile |
| Vgr-1 | ... | ... | Val | Met | ... | ... | ... | Tyr | ... |
| CBMP-2A | ... | Asn | Ser | Val | ... | Ser | ... | Lys | Ile |
| CBMP-2B | ... | Asn | Ser | Val | ... | Ser | ... | Ser | Ile |
| BMP3 | ... | Arg | Ala* | Gly | Val | Val | Pro | Gly | Ile |
| GDF-1 | Met | ... | Ala | Ala | Ala | ... | Gly | Ala | Ala |
| 60A | ... | ... | Leu | Leu | Glu | ... | Lys | Lys | ... |
| BMP5 | ... | ... | Leu | Met | Phe | ... | Asp | His | ... |
| BMP6 | ... | ... | Leu | Met | ... | ... | ... | Tyr | ... |
| | | | | | | 55 | 60 | | |

FIGURE 7-7

| | Pro | Lys | Pro | Cys | Cys | Ala | Pro | Thr | Gln |
|---------|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| hOP-1 | ... | ... | ... | ... | ... | ... | ... | ... | ... |
| mOP-1 | ... | ... | ... | Ala | ... | ... | ... | ... | Lys |
| hOP-2 | ... | ... | ... | Ala | ... | ... | ... | ... | ... |
| mOP-2 | ... | ... | ... | Ala | ... | ... | ... | ... | Lys |
| mOP-3 | ... | ... | ... | Val | ... | Val | ... | ... | Glu |
| DPP | ... | ... | ... | Ala | ... | Val | ... | ... | ... |
| Vgl | ... | ... | Leu | ... | ... | Val | ... | ... | Lys |
| Vgr-1 | ... | ... | ... | ... | ... | ... | ... | ... | Lys |
| CBMP-2A | ... | ... | Ala | ... | ... | Val | ... | ... | ... |
| CBMP-2B | ... | ... | Ala | ... | ... | Val | ... | ... | Glu |
| BMP3 | ... | Glu | ... | ... | ... | Val | ... | ... | Glu |
| GDF-1 | Asp | Leu | ... | ... | ... | Val | ... | Ala | Lys |
| 60A | ... | ... | ... | ... | ... | ... | ... | Arg | Arg |
| BMP5 | ... | ... | ... | ... | ... | ... | ... | ... | ... |
| BMP6 | ... | ... | ... | ... | ... | ... | ... | ... | Lys |
| | | | | | | | | | 70 |
| | | | | | | | | | 65 |

FIGURE 7-8

| | | | | | | | | | |
|---------|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| hOP-1 | Leu | Asn | Ala | Ile | Ser | Val | Leu | Tyr | Phe |
| mOP-1 | ... | ... | ... | ... | ... | ... | ... | ... | ... |
| hOP-2 | ... | Ser | ... | Thr | ... | ... | ... | ... | Tyr |
| mOP-2 | ... | Ser | ... | Thr | ... | ... | ... | ... | Tyr |
| mOP-3 | ... | Ser | ... | ... | ... | Leu | ... | ... | Tyr |
| Vgl | Met | Ser | Pro | ... | Met | ... | Phe | Tyr | |
| Vgr-1 | Val | ... | ... | ... | ... | ... | ... | ... | ... |
| DPP | ... | Asp | Ser | Val | Ala | Met | ... | ... | Leu |
| CBMP-2A | ... | Ser | ... | ... | ... | Met | ... | ... | Leu |
| CBMP-2B | ... | Ser | ... | ... | ... | Met | ... | ... | Leu |
| BMP3 | Met | Ser | Ser | Leu | ... | Ile | ... | Phe | Tyr |
| GDF-1 | ... | Ser | Pro | ... | ... | ... | ... | Phe | ... |
| 60A | ... | Gly | ... | Leu | Pro | ... | ... | ... | His |
| BMP5 | ... | ... | ... | ... | ... | ... | ... | ... | |
| BMP6 | ... | ... | ... | ... | ... | ... | ... | ... | |
| | | | | | 75 | | 80 | | |

FIGURE 7-9

| | Asp | Asp | Ser | Ser | Asn | Val | Ile | Leu | Lys |
|---------|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| hOP-1 | Asp | Asp | ... | ... | ... | ... | ... | ... | ... |
| mOP-1 | ... | ... | ... | ... | ... | ... | ... | ... | ... |
| hOP-2 | ... | Ser | ... | Asn | ... | ... | ... | ... | Arg |
| mOP-2 | ... | Ser | ... | Asn | ... | ... | ... | ... | Arg |
| mOP-3 | ... | Arg | Asn | Asn | ... | ... | ... | ... | Arg |
| DPP | Asn | ... | Gln | ... | Thr | ... | Val | ... | ... |
| Vgl | ... | Asn | Asn | Asp | ... | ... | Val | ... | ... |
| Vgr-1 | ... | ... | Asn | ... | ... | ... | ... | ... | Arg |
| CBMP-2A | ... | Glu | Asn | Glu | Lys | ... | Val | ... | ... |
| CBMP-2B | ... | Glu | Tyr | Asp | Lys | ... | Val | ... | ... |
| BMP3 | ... | Glu | Asn | Lys | ... | ... | Val | ... | ... |
| GDF-1 | ... | Asn | ... | Asp | ... | ... | Val | ... | ... |
| 60A | Leu | Asn | Asp | Glu | ... | ... | Asn | ... | ... |
| BMP5 | ... | ... | ... | ... | ... | ... | ... | ... | ... |
| BMP6 | ... | ... | Asn | ... | ... | ... | ... | ... | ... |

| | Lys | Tyr | Arg | Asn | Met | Val | Val | Arg |
|---------|-----|-----|-----|-----|-----|-----|-----|-----|
| hOP-1 | ... | ... | ... | ... | ... | ... | ... | ... |
| mOP-1 | ... | ... | His | ... | ... | ... | ... | Lys |
| hOP-2 | ... | ... | His | ... | ... | ... | ... | Lys |
| mOP-2 | ... | ... | His | ... | ... | ... | ... | Lys |
| mOP-3 | Arg | Glu | ... | ... | ... | ... | ... | Gln |
| DPP | Asn | ... | Gln | Glu | ... | Thr | ... | Val |
| Vgr1 | His | ... | Glu | ... | ... | Ala | ... | Asp |
| Vgr-1 | ... | ... | ... | ... | ... | ... | ... | ... |
| CBMP-2A | Asn | ... | Gln | Asp | ... | ... | ... | Glu |
| CBMP-2B | Asn | ... | Gln | Glu | ... | ... | ... | Glu |
| BMP3 | Val | ... | Pro | ... | ... | Thr | ... | Glu |
| GDF-1 | Gln | ... | Glu | Asp | ... | ... | ... | Asp |
| 60A | ... | ... | ... | ... | ... | Ile | ... | Lys |
| BMP5 | ... | ... | ... | ... | ... | ... | ... | ... |
| BMP6 | ... | ... | ... | TrP | ... | ... | ... | ... |
| | | | | | | | 95 | |

FIGURE 7-11

| | | | | | |
|---------|-----|-----|-----|-----|-----|
| hOP-1 | Ala | Cys | Gly | Cys | His |
| mOP-1 | ... | ... | ... | ... | ... |
| hOP-2 | ... | ... | ... | ... | ... |
| mOP-2 | ... | ... | ... | ... | ... |
| mOP-3 | ... | ... | ... | ... | ... |
| DPP | Gly | ... | ... | ... | Arg |
| Vgr-1 | Glu | ... | ... | ... | Arg |
| CBMP-2A | Gly | ... | ... | ... | Arg |
| CBMP-2B | Gly | ... | ... | ... | Arg |
| BMP3 | Ser | ... | Ala | ... | Arg |
| GDF-1 | Glu | ... | ... | ... | Arg |
| 60A | Ser | ... | ... | ... | ... |
| BMP5 | Ser | ... | ... | ... | ... |
| BMP6 | ... | ... | ... | ... | ... |
| | | | | | 100 |

* Between residues 56 and 57 of BMP3 is a Val residue;
between residues 43 and 44 of GDF-1 lies the amino acid
sequence Gly-Gly-Pro-Pro.

FIGURE 7-12

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 97/07816

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 A61K38/18

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 A61K C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|----------|--|-----------------------|
| A | WO 93 05751 A (CREATIVE BIOMOLECULES, INC) 1 April 1993 see the whole document --- | 1-60 |
| A | WO 94 06449 A (CREATIVES BIOMOLECULES, INC) 31 March 1994 see the whole document --- | 1-60 |
| P,X | 29TH ANNUAL MEETING OF THE AMERICAN SOCIETY OF NEPHROLOGY, NEW ORLEANS, LOUISIANA, USA, NOVEMBER 3-6, 1996. JOURNAL OF THE AMERICAN SOCIETY OF NEPHROLOGY 7 (9). 1996. 1867, XP002038677 VUKICEVIC S ET AL: "Recombinant human OP-1 (BMP-7) prevents rapid loss of glomerular function and improves mortality associated with chronic renal failure." See abstract A3102 ----- | 1-60 |

Further documents are listed in the continuation of box C.

Patent family members are listed in annex.

* Special categories of cited documents:

- 'A' document defining the general state of the art which is not considered to be of particular relevance
- 'E' earlier document but published on or after the international filing date
- 'L' document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- 'O' document referring to an oral disclosure, use, exhibition or other means
- 'P' document published prior to the international filing date but later than the priority date claimed

- 'T' later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- 'X' document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- 'Y' document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
- '&' document member of the same patent family

1

Date of the actual completion of the international search

Date of mailing of the international search report

8 September 1997

17.09.97

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentstaan 2
NL - 2280 HV Rijswijk
Tel. (+31-70) 340-2040, Tx. 31 651 epo nl
Fax (+31-70) 340-3016

Authorized officer

Moreau, J

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 97/07816

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. Claims Nos.: **1-60**

because they relate to subject matter not required to be searched by this Authority, namely:

Remark: Although claim(s) 1 to 60

is(are) directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2.

Claims Nos.:

because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3.

Claims Nos.:

because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

The additional search fees were accompanied by the applicant's protest.

No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 97/07816

| Patent document cited in search report | Publication date | Patent family member(s) | | Publication date |
|--|------------------|-------------------------|--|------------------|
| WO 9305751 A | 01-04-93 | AU 669127 B | | 30-05-96 |
| | | AU 2564592 A | | 05-04-93 |
| | | AU 670558 B | | 25-07-96 |
| | | AU 3176293 A | | 27-04-93 |
| | | CA 2104678 A | | 12-09-92 |
| | | CA 2116559 A | | 01-04-93 |
| | | CA 2116562 A | | 18-03-93 |
| | | EP 0601106 A | | 15-06-94 |
| | | EP 0601135 A | | 15-06-94 |
| | | JP 6510989 T | | 08-12-94 |
| | | JP 7502021 T | | 02-03-95 |
| | | WO 9304692 A | | 18-03-93 |
| | | US 5656593 A | | 12-08-97 |
| | | US 5650276 A | | 22-07-97 |
| | | US 5652337 A | | 29-07-97 |
| | | US 5652118 A | | 29-07-97 |
| | | AU 678345 B | | 29-05-97 |
| | | AU 2862492 A | | 05-04-93 |
| | | CA 2116560 A | | 18-03-93 |
| | | EP 0601129 A | | 15-06-94 |
| | | JP 6510432 T | | 24-11-94 |
| | | WO 9305172 A | | 18-03-93 |
| | | AU 678380 B | | 29-05-97 |
| | | AU 4795193 A | | 03-03-94 |
| | | AU 673006 B | | 24-10-96 |
| | | AU 4995593 A | | 03-03-94 |
| | | CA 2141555 A | | 17-02-94 |
| | | CA 2141556 A | | 17-02-94 |
| | | EP 0652953 A | | 17-05-95 |
| | | EP 0661933 A | | 12-07-95 |
| | | JP 7509611 T | | 26-10-95 |
| | | JP 7509720 T | | 26-10-95 |
| | | WO 9403600 A | | 17-02-94 |
| | | WO 9403075 A | | 17-02-94 |
| ----- | | | | |
| WO 9406449 A | 31-03-94 | AU 678380 B | | 29-05-97 |
| | | AU 4795193 A | | 03-03-94 |
| | | AU 4797193 A | | 03-03-94 |
| | | AU 4995593 A | | 03-03-94 |
| | | AU 5129293 A | | 12-04-94 |

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 97/07816

| Patent document cited in search report | Publication date | Patent family member(s) | Publication date |
|--|------------------|-------------------------|------------------|
| WO 9406449 A | | AU 5129393 A | 12-04-94 |
| | | AU 5162393 A | 12-04-94 |
| | | AU 5290893 A | 12-04-94 |
| | | AU 5590094 A | 24-05-94 |
| | | CA 2141554 A | 17-02-94 |
| | | CA 2141555 A | 17-02-94 |
| | | CA 2141556 A | 17-02-94 |
| | | CA 2147598 A | 11-05-94 |
| | | EP 0652953 A | 17-05-95 |
| | | EP 0653942 A | 24-05-95 |
| | | EP 0661933 A | 12-07-95 |
| | | EP 0665739 A | 09-08-95 |
| | | EP 0661987 A | 12-07-95 |
| | | EP 0680334 A | 08-11-95 |
| | | EP 0672064 A | 20-09-95 |
| | | JP 7509611 T | 26-10-95 |
| | | JP 7509720 T | 26-10-95 |
| | | JP 7509721 T | 26-10-95 |
| | | JP 8501779 T | 27-02-96 |
| | | JP 8501558 T | 20-02-96 |
| | | JP 8501315 T | 13-02-96 |
| | | JP 8503198 T | 09-04-96 |
| | | WO 9403600 A | 17-02-94 |
| | | WO 9403075 A | 17-02-94 |
| | | WO 9403200 A | 17-02-94 |
| | | WO 9406447 A | 31-03-94 |
| | | WO 9406399 A | 31-03-94 |
| | | WO 9406420 A | 31-03-94 |
| | | WO 9410203 A | 11-05-94 |
| | | US 5652337 A | 29-07-97 |
| | | US 5652118 A | 29-07-97 |